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Inhalation Exposure to Methyl *tert*-Butyl Ether (MTBE) Using Continuous Breath Analysis

by

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Foreword

The mission of the National Exposure Research Laboratory (NERL) is to provide scientific understanding, information, and assessment tools that will quantify and reduce the uncertainty in EPA's exposure and risk assessments for environmental stressors. These stressors include chemicals, biologicals, radiation, and changes in climate, land use, and water use. The Laboratory's primary function is to measure, characterize, and predict human and ecological exposure to pollutants. Exposure assessments are integral elements in the risk assessment process used to identify populations and ecological resources at risk. The EPA relies increasingly on the results of quantitative risk assessments to support regulations, particularly of chemicals in the environment. In addition, decisions on research priorities are influenced increasingly by comparative risk assessment analysis. The utility of the risk-based approach, however, depends on accurate exposure information. Thus, the mission of NERL is to enhance the Agency's capability for evaluating exposure of both humans and ecosystems from a holistic perspective.

The National Exposure Research Laboratory focuses on four major research areas: predictive exposure modeling, exposure assessment, monitoring methods, and environmental characterization. Underlying the entire research and technical support program of the NERL is its continuing development of state-of-the-art modeling, monitoring, and quality assurance methods to assure the conduct of defensible exposure assessments with known certainty. The research program supports its traditional clients – Regional Offices, Regulatory Program Offices, ORD Offices, and Research Committees – and ORD's Core Research Program in the areas of health risk assessment, ecological risk assessment, and risk reduction.

Monitoring techniques for volatile organic compounds (VOCs) in air or exhaled breath are constantly evolving as the needs of the exposure assessment and health effects communities change. The continuous real-time breath analyzer provides a unique means of collecting abundant data with which to track the uptake, distribution in the body, and decay of numerous compounds of interest to NERL. The purpose of the present study was to better understand the uptake and disposition of methyl *t*-butyl ether (MTBE) and dibromochloromethane (DBCM) within the human body as a result of inhalation exposure.

Gary J. Foley
Director
National Exposure Research Laboratory

Abstract

The oxygenate methyl *tert*-butyl ether (MTBE) has been added to gasoline to meet national ambient air quality standards in those parts of the U.S. that are non-compliant for carbon monoxide. Although MTBE has provided important health benefits in terms of reduced hazardous air pollutants, the increasing occurrence and detection of MTBE in drinking water sources in California, New Jersey, and elsewhere has raised concerns about potential exposures from water usage and resulting health effects. In addition to MTBE, disinfection byproducts can be present in the water people use for showering, bathing, or drinking, as a result of the reaction of disinfection agents with organic material already present in water. Chlorine reacts with humic acids to form the trihalomethanes, which are the most common and abundant byproducts in chlorinated water. Besides chloroform, which has been widely studied, the byproduct dibromochloromethane (DBCM) occurs as a result of the chlorination process in those areas that naturally have bromide in their ground water. Relatively little information on exposure to this chemical is available.

This study was designed to determine the uptake by humans of MTBE and DBCM as a result of controlled, short-term inhalation exposures. Our approach made use of continuous real-time breath analysis to generate exhaled-breath profiles, and evaluate MTBE and DBCM kinetics in the body. Seven subjects were exposed continuously via face mask to 2,217 $\mu g/m^3$ (542 ppbv) MTBE-d_{12} and 728 $\mu g/m^3$ (85.6 ppbv) DBCM, except for several brief (~2-min) intervals during which breath measurements were taken. Total exposure time was ~30 min, followed by exposure to clean air for a further 30 — 60 min. Exhaled breath was sampled and analyzed with the real-time breath technology; blood samples were simultaneously collected from the subjects (3-4 samples during exposure; 2-5 samples post-exposure). The real-time technology was specially modified with a biofeedback exposure control system to allow us to make uptake measurements during the exposure period; breath measurements were taken continuously throughout the post-exposure period.

The exposures resulted in an increase in the measured breath concentration of MTBE-d_{12} from pre-exposure levels of 10 – 20 $\mu g/m^3$ (2 – 5 ppbv) to 200 – 450 $\mu g/m^3$ (50 – 110 ppbv) following exposure. MTBE-d_{12} blood concentrations increased from the limit of detection, 0.30 $\mu g/L$, to ~0.9 – 2.5 $\mu g/L$ at the end of the ~30-min exposure period.

The time-course measurements of both exhaled breath and venous blood are well-described by the linear compartmental uptake and elimination models, the interpretation of which provides important information on the residence times of the compound in the body, the relative capacity of each compartment, and the fraction of the chemical exhaled unchanged at equilibrium. The breath uptake data were consistent with a one-compartment model. The mean

value for the one-compartment uptake residence times $\tau_{1uptake}$ was 5.7 ± 2.4 (SD) min (range 3.3 – 9.8 min). In contrast, the breath decay phase data gave satisfactory two-compartment fits. The mean value for the first compartment decay residence times τ_{1decay} was 3.8 ± 1.9 (SD) min (range 2.4 – 7.8 min); for the second compartment, the mean decay residence time τ_{2decay} was 61 ± 11 (SD) min (range 46 – 73 min). The blood uptake data were also consistent with a one-compartment model and were convergent in almost all cases. The average blood uptake residence time was essentially the same as that for the breath. The quality of the blood decay data were such that we were only able to extract meaningful information from 2 or 3 data sets.

The mean MTBE-d_{12} total absorbed ("internal") dose was 149 ± 34 µg for the average 30-min exposure and a mean total ("applied") dose of 209 µg. The mean fraction of MTBE-d_{12} absorbed, or relative uptake, was 0.73 ± 0.04. The mean value for *f*, the fraction of the MTBE-d_{12} exposure concentration exhaled unchanged was 0.29 ± 0.04. This value is in good agreement with the value recently reported by Lee et al. Using linear regression analysis, the mean blood/breath ratio for MTBE-d_{12} was found to be 6.7 ± 3.4. This value is significantly lower than values obtained in previous studies. The reason for this discrepancy is not clear.

By and large, background levels for DBCM in the exhaled breath were below the limit of detection, and the signal measured for this compound at m/z 129, the most abundant ion in the glow discharge mass spectrum, was exceptionally "noisy". The average signals during the uptake phase provided initial (pre-exposure) breath concentrations that ranged from 70 to 160 µg/m^3 and rose to between 130 and 250 µg/m^3 after 30 minutes. The high initial breath concentrations suggest that the measured signal at m/z 129 was probably elevated due to an unknown contaminant with fragment ions at the same mass. For TBA, all of the blood measurements were below the detection limit.

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Acknowledgments

We thank Dr. Lance A. Wallace of U.S. EPA for his expert advice and suggestions throughout this investigation. Major contributions to the research effort were made by Battelle personnel Marielle C. Brinkman and Jan Satola.

Chapter 1
Introduction

Methyl *tert*-butyl ether (MTBE) was first introduced in the U.S. as a synthetic gasoline additive in the 1970s. The federal Clean Air Act requirements for oxygenates in wintertime gasoline made MTBE, which has oxygen-containing properties, a popular choice of refineries manufacturing reformulated gasoline. Added to gasoline at levels of up to 15% by volume, MTBE reduces automotive emissions of carbon monoxide.

A survey of ground water throughout the United States by the U.S. Geological Survey has indicated that MTBE is one of the most frequently detected compounds in ground water.[1] MTBE is highly water-soluble and appears to be resistant to chemical and microbial degradation in water.[2] When MTBE, which has a very unpleasant taste and odor,[3] began appearing in groundwater and some public drinking water systems throughout the U.S., environmental agencies, state governments, and regulatory groups became concerned. Issues of toxicology and exposure during automobile refueling also pointed to the need for information on the exposure levels and distribution of MTBE in the human body.

Besides MTBE, the trihalomethanes (chloroform, bromodichloromethane, dibromochloro-methane, bromoform) can be present in the water people use for showering, bathing, or drinking, if the water supply was disinfected with chlorine and contaminated with MTBE. The most common method of disinfecting water in the U.S. is by adding chlorine directly to the water. Disinfection byproducts (DBPs) result from the reaction of disinfection agents with organic material already present in water. Chlorine reacts with humic acids to form the trihalomethanes, the haloacetic acids, and many other halogenated compounds. Of the many classes of disinfection byproducts that occur, trihalomethanes are the most common and abundant in chlorinated water. The DBP, dibromochloromethane (DBCM) occurs in the chlorination process in those areas that naturally have bromide in their ground water. Dibromochloromethane has been reported to occur at about 40 μg/L at the 90th percentile in Los Angeles, CA[4].

Exposure to MTBE can occur by inhalation, dermal contact, or ingestion.[5] Vehicle refueling activities lead to the highest potential exposures by inhalation, with breathing zone levels ranging from 0.1 to 4 ppm for 1 – 2 min durations and peaks occasionally exceeding 10 ppm.[6,7,8] The health effects of exposures to gasoline or water containing MTBE are not well-established, although acute effects such as headaches, nausea or vomiting, nasal and ocular irritation, and sensations of disorientation, have been associated with exposure to gasoline containing MTBE.[9,10] In those areas of the U.S. that use MTBE as a gasoline oxygenate, doses from non-occupational exposure are between 0.4 and 6 μg/kg-day, and roughly 1.4 μg/kg-day as a result of exposure via contaminated water.[11]

The uptake of MTBE by inhalation has been measured in exhaled breath under controlled conditions using integrated sampling techniques.[7,12,13,14,15,16] Several studies, including some based on the analysis of exhaled breath, have demonstrated significant dermal absorption of chloroform and trichloroethylene while showering or bathing, and the dose is roughly comparable to that resulting from inhalation.[17,18,19,20,21,22,23,24] Because of the dynamic equilibrium between the concentration of a VOC in the blood and its concentration in exhaled breath,[25] breath measurements can be used to estimate body burden and to detect changes in body burden with time.[26,27,28,29] Most previous measurements of human breath concentrations of VOCs to determine the dose resulting from inhalation exposure to the pollutant in air have, however, relied on the use of integrated sampling methods and subsequent batch analysis. This has limited the number of samples that are typically collected in such exposure studies to about four during the uptake phase and usually no more than about twelve during the decay phase, thus reducing the reliability of data designed to address these issues.[30]

Several recent studies conducted at Battelle under the auspices of the U.S. Environmental Protection Agency (EPA) have demonstrated the value of using continuous breath measurements to determine exposure to volatile organic compounds (VOCs).[30,31,32] This monitoring technology, based on direct breath sampling coupled with mass spectrometry, offers a powerful means of extracting VOCs directly from the breath matrix and eliminates the pre-concentration step that normally precedes exhaled air analysis by conventional gas chromatography/mass spectrometry (GC/MS).[27,33] The real-time breath measurement method provides abundant data, and thus better time resolution over the uptake and elimination periods of an exposure episode, compared to previous discrete time-integrated breath sampling methods.[21,27,28,31,32,33,34,35,36]

We have used the breath analysis technology to measure dermal absorption to chloroform while bathing or showering, as well as exposure to the chemical by inhalation.[21,37] Showering or bathing in water contaminated with chloroform gives rise to a measurable dose of chloroform through dermal exposures. We showed that water temperature has a powerful effect on dermal absorption of chloroform while bathing, with about a 30-fold increase in absorbed chloroform occurring over a 10°C increase in bath water temperature.[21] The inhalation measurements provided important information on the residence times of the compound in the body, the relative capacity of each compartment, and the fraction of the chemical exhaled unchanged at equilibrium.[37]

The purpose of the present study was to use the real-time breath measurement technology to determine more precisely than previous studies the residence times in various physiological compartments for MTBE in blood and breath. The study was also designed to provide analogous data on DBCM and on the blood/breath ratios for MTBE and DBCM. A secondary purpose was to analyze the data in an attempt to develop a model for MTBE that allows for the inclusion of a mucous membrane component, if appropriate, since previous work has suggested that this may be an important component of MTBE distribution in the body.[12]

Chapter 2
Conclusions

The oxygenate methyl *tert*-butyl ether (MTBE) has been added to gasoline to meet national ambient air quality standards in those parts of the U.S. that are non-compliant for carbon monoxide. Although MTBE has provided important health benefits in terms of reduced hazardous air pollutants, the increasing occurrence and detection of MTBE in drinking water sources in California, New Jersey, and elsewhere has raised concerns about potential exposures from water usage and resulting health effects. In addition to MTBE, disinfection byproducts can be present in the water people use for showering, bathing, or drinking, as a result of the reaction of disinfection agents with organic material already present in water. Chlorine reacts with humic acids to form the trihalomethanes, which are the most common and abundant byproducts in chlorinated water. Besides chloroform, which has been widely studied, the byproduct dibromochloromethane (DBCM) occurs as a result of the chlorination process in those areas that naturally have bromide in their ground water. Relatively little information on exposure to this chemical is available. The purpose of this study was to measure directly the uptake by humans of MTBE and DBCM as a result of controlled, short-term inhalation exposures. Simultaneous blood samples were also collected and analyzed as part of the study.

Seven subjects were exposed continuously via face mask to 2,217 $\mu g/m^3$ (542 ppbv) MTBE-d_{12} and 728 $\mu g/m^3$ (85.6 ppbv) DBCM, except for several brief (~2-min) intervals during which breath measurements were taken. Total exposure time was ~30 min, followed by exposure to clean air for a further 30 — 60 min. Exhaled breath was sampled and analyzed with the real-time breath technology; blood samples were simultaneously collected from the subjects (3-4 samples during exposure; 2-5 samples post-exposure) and analyzed separately for MTBE-d_{12} and DBCM, as well as for the MTBE metabolite, *t*-butyl alcohol. The real-time technology was specially modified with a biofeedback exposure control system to allow us to make uptake measurements during the exposure period; breath measurements were taken continuously throughout the post-exposure period. The uptake and decay of the target chemicals in the blood was estimated by fitting the exposure and post-exposure breath and blood data to a linear multi-compartmental model that estimated residence times. The measurements also provided information on blood:breath concentration ratios, as well as the fraction of breath MTBE and DBCM exhaled unchanged at equilibrium. The exposures resulted in an increase in the measured breath concentration of MTBE-d_{12} from pre-exposure levels of 10 – 20 $\mu g/m^3$ (2 – 5 ppbv) to 200 – 450 $\mu g/m^3$ (50 – 110 ppbv) following exposure. MTBE-d_{12} blood concentrations increased from the limit of detection, 0.30 $\mu g/L$, to ~0.9 – 2.5 $\mu g/L$ at the end of the ~30-min exposure period.

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Using linear regression analysis, the mean blood/breath ratio for MTBE-d_{12} was found to be 6.7 ± 3.4. This value is significantly lower than values obtained in previous studies. The reason for this discrepancy is not clear.

By and large, background levels for DBCM in the exhaled breath were below the limit of detection, and the signal measured for this compound at m/z 129, the most abundant ion in the glow discharge mass spectrum, was exceptionally "noisy". The average signals during the uptake phase provided initial (pre-exposure) breath concentrations that ranged from 70 to 160 $\mu g/m^3$ and rose to between 130 and 250 $\mu g/m^3$ after 30 minutes. The high initial breath concentrations suggest that the measured signal at m/z 129 was probably elevated due to an unknown contaminant with fragment ions at the same mass. For TBA, all of the blood measurements were below the detection limit.

Chapter 3
Recommendations

The real-time breath analyzer is a powerful technique for obtaining unique data on VOCs in human exhaled breath in situations in which the concentrations of the constituents change rapidly. In the present study, it was used to determine the uptake by humans of MTBE-d_{12} and DBCM as a result of controlled, short-term inhalation exposure.

Analysis problems experienced at EOHSI prevented us from obtaining reliable data for the target compounds in blood and urine. These biological measurements are important, complementary information which serve to provide a more complete picture of the uptake, distribution, and elimination of the pollutants from the body than is available from an analysis of the exhaled breath data alone. It is recommended that careful attention be paid in future studies to first ensuring that the analytical techniques for the characterization of target analytes in blood and urine are reliable and can be applied without difficulty before embarking on similar studies.

Chapter 4
Experimental Procedures

In this scenario, subjects wore a full face mask and were exposed by inhalation only to a precisely measured amount of isotopically-labeled MTBE and dibromochloromethane (DBCM). Following exposure, the subjects inhaled pure air and the exhaled breath was monitored for a period to obtain residence times of chloroform in the body compartments. Pre-exposure, exposure, and post-exposure blood samples were drawn at the same time as the breath was being monitored, and the blood samples were analyzed separately for MTBE and DBCM. All inhalation experiments were conducted in a bathroom at the Environmental and Occupational Health Sciences Institute (EOHSI), Rutgers University, in Piscataway, NJ.

Experimental Procedures

Subject Selection and Recruitment

Volunteers for the study were sought from amongst the student population at Rutgers University, in Piscataway, NJ by means of notices placed in buildings around the University campus and local newspaper advertisements. Respondents with any of the following medical conditions were excluded: neurologic disease or brain injury, significant exposure to other neurotoxicants, chronic fatigue syndrome or multiple chemical sensitivity, stroke or cardio-vascular disease, serious pulmonary disease, liver or kidney disease, serious gastrointestinal disorders (e.g. colitis), claustrophobia, and major psychiatric conditions including psychoses, manic depression, alcoholism, or drug abuse. No pregnant or lactating women were included.

The subjects were healthy, young nonsmoker adults of average weight and height. Information on the subjects is provided in Table 4-1 along with a summary of the exposure conditions. Information was collected from each subject on his/her age, height, weight, respiration rate (using a dry gas meter), and percent body fat (from body circumferences and height). The study protocol was reviewed and approved by both the Battelle Human Subjects Committee and the EOHSI Institutional Review Board (IRB), then was approved by the EPA Human Subjects Committee. Informed written consent was obtained from each subject before participation. Each subject received financial compensation on completion of the exposure experiments.

Exposure Conditions

According to the Integrated Household Exposure Model,[38] a water concentration of 200 μg/L would be expected to result in a shower air concentration of 0.5 ppm for a fifteen minute shower. For the U.S. population, the 90th percentile duration for showering is 30 minutes.[39]

Table 4-1. Characteristics of subjects who participated in inhalation exposure study at EOHSI, and associated exposure conditions

Subject	Sex[a]	Height (cm)	Weight (kg)	Age (yr)	Expt. Date	MTBE-d_{12}/DBCM Concn. in Air ($\mu g/m^3$)	Exposure Duration (min)	RTBA Sample ID	Calibration File ID
IF02	F	163	58.1	21	02/19/01	2,217/728	33.5	IF02; IF02b	cal0219a
IM03	M	185	90.7	36	02/20/01	2,217/728	36.7	IM03; IM03b; IM03c	cal0220a
IM04	M	173	61.2	21	02/21/01	2,217/728	33.5	IM04; IM04b; IM04c	cal0221a; cal0221b; cal0221c; cal0221d
IM05	M	175	83.9	54	02/21/01	2,217/728	33.9	IM05; IM05b	
IM08	M	173	79.4	26	02/22/01	2,217/728	35.8	IM08R; IM08Rb; IM08Rc	cal0222a; cal0222b
IF06	F	163	52.6	19	02/22/01	2,217/728	33.8	IF06; IF06b	
IM01	M	170	77.1	22	02/23/01	2,217/728	34.2	IM01; IM01b	cal0223a

[a] Abbreviations: M, male; F, female.

The subjects were, consequently, exposed for 30 minutes to a mixture of 2,217 $\mu g/m^3$ (542 ppbv) MTBE-d_{12} and 728 $\mu g/m^3$ (85.6 ppbv) DBCM in humidified zero-grade air. In previous work done at EOHSI, subjects were exposed to 1 ppm MTBE in a gasoline mixture for 15 minutes to simulate exposures that occur during fueling of automobiles.[15,16] The nominal exposure of 0.5 ppm MTBE for 30 minutes was selected here to represent an equivalent dose.

Gas mixtures for inhalation exposure were prepared in pressurized aluminum gas cylinders and consisted of 0.5 ppm isotopically-labeled MTBE-d_{12} (>99.8 atom % D; Lot No. F65P1; C/D/N Isotopes; CAS No. 29366-08-3) and 0.12 ppm DBCM in humidified zero-grade air. In order to ensure that the subject was exposed to a precisely metered amount of the chemical, the cylinder containing the gas mixture was attached to the closed delivery system shown schematically in Figure 4-1. The inlet tube to the full face mask (Hans Rudolph Model 8932) was attached to the cylinder and discrete amounts of MTBE-d_{12} and DBCM in the air stream flowed to the subject with each inhalation through the full face mask on a demand basis. The amounts of the chemicals inhaled with each inspiration were registered incrementally by means of a dry gas meter (Model DTM-115, American Meter Co.), which was attached to the vent of the breath inlet system via wide-bore flexible tubing. The dry gas meter also recorded the total amount inspired over the entire exposure period and the respiration rate of each subject was monitored. The total amount of the chemical exhaled unchanged was obtained from the area under the breath concentration/time curve. The MTBE-d_{12} and DBCM concentrations in the

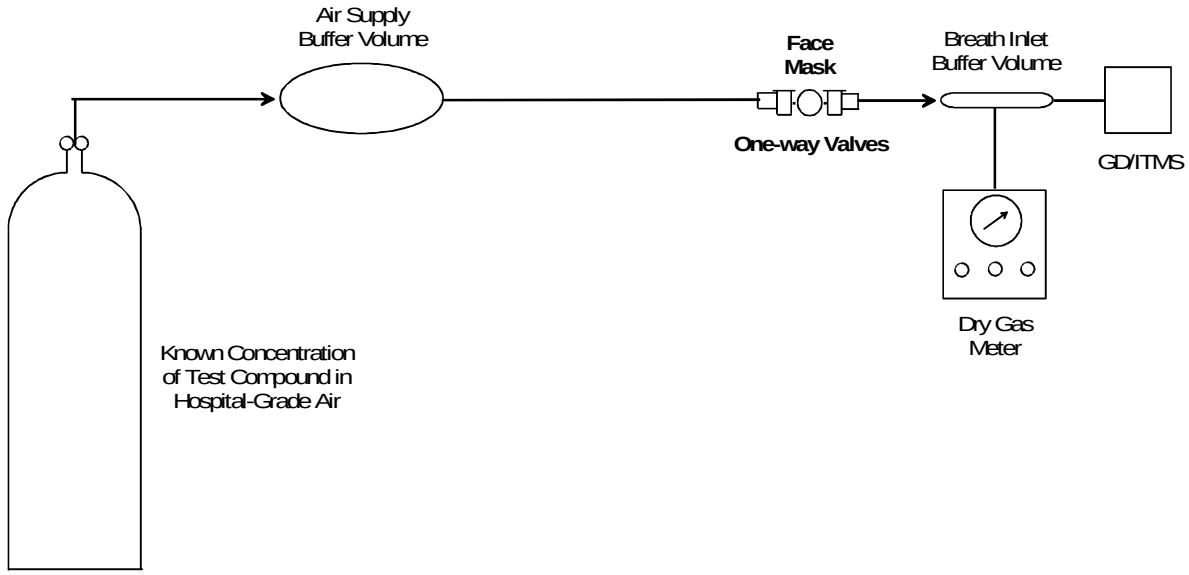

Figure 4-1. Closed delivery system to (i) provide subject wearing full face mask with precisely metered amount of chemical(s) for inhalation (from pressurized gas cylinder and dry gas meter); and (ii) to measure amount of chemical exhaled unchanged (via dry gas meter attached to breath interface and (glow discharge/ion trap mass spectrometer) breath analyzer).

cylinder were monitored by taking 6-L samples from the cylinder in evacuated stainless steel canisters and analyzing them by a modified U.S. EPA Method TO-14.[40]

At the end of the exposure, the subject was switched to a pure air supply, and real-time breath measurements continued uninterruptedly for a further 30 to 60 minutes. Then, periodically during the next hour, the subject provided further breath samples for periods of 5-10 minutes each until the concentrations approached the pre-exposure levels.

Sampling and Measurement Procedures

Breath

To conduct these studies and ensure that the uptake of the target chemicals could be monitored in real time, we developed an automated system for use with the real-time breath analyzer (RTBA).

The breath analyzer, shown schematically in Figure 4-2, consists of a Battelle-patented breath inlet unit, a direct breath sampling interface (glow discharge ionization source), and an ion trap mass spectrometer (ITMS). A face mask (Hans Rudolph Model 8932) equipped with a two-way non-rebreathing valve set is attached to the breath inlet. As shown in Figure 4-3, the inlet to the face mask is connected to the MTBE-d_{12}/DBCM standard exposure source (gas cylinder) or a source of hospital grade breathing air through a 3-way wide-bore pneumatic solenoid valve. The outlet from the face mask is attached to the holding volume of the breath inlet through a second 3-way wide-bore pneumatic solenoid valve. When the breath holding volume is connected to the analyzer through the third 3-way solenoid valve, the breath sample is vacuum-extracted at a constant rate by the vacuum pump of the glow discharge source and flows into the ion trap without any attention from the subject.

The volume of the breath inlet (in Figure 4-2) is normally less than 100 mL, or roughly one-fifth the mean value of the adult tidal volume. Under these conditions, each breath exhalation effectively displaces the previous breath sample while a steady gas flow is maintained into the analyzer. This ensures that unit resolution is achieved between individual breath exhalations while at the same time producing a constant and undiluted sample for analysis. A dry gas meter (Model DTM-115, American Meter Co.), attached to the vent of the breath inlet system via wide-bore flexible tubing, was used to record the respiration rate and total exhaled volume from each subject.

The direct breath sampling interface is a glow discharge ionization source, which is attached to the ITMS. The operation of this system has been described in detail elsewhere.[41,42,43] For this study, we used a Teledyne Electronic Technologies (Mountain View, CA) 3DQ™ Discovery ion trap MS as the analyzer.[44] The 3DQ is a compact, field-deployable instrument with high sensitivity and specificity. The breath analyzer was set up to measure the MTBE-d_{12} and DBCM target analytes both in the single MS as well as the MS/MS mode. The ions selected for this purpose are listed in Table 4-2. Calibration measurements conducted in our laboratory showed that MTBE-d_{12} can be determined in humidified air with high sensitivity and specificity.

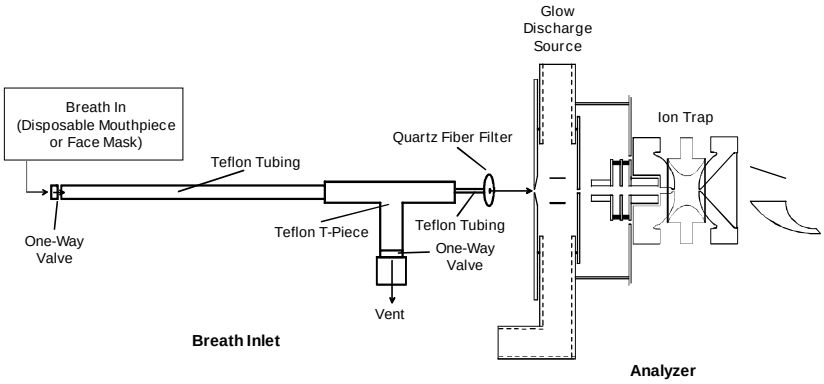

Figure 4-2. Continuous real-time breath analyzer (RTBA), consisting of breath inlet (breath holding volume) attached to direct breath sampling interface (glow discharge ionization source) and ion trap mass spectrometer (GD/ITMS).

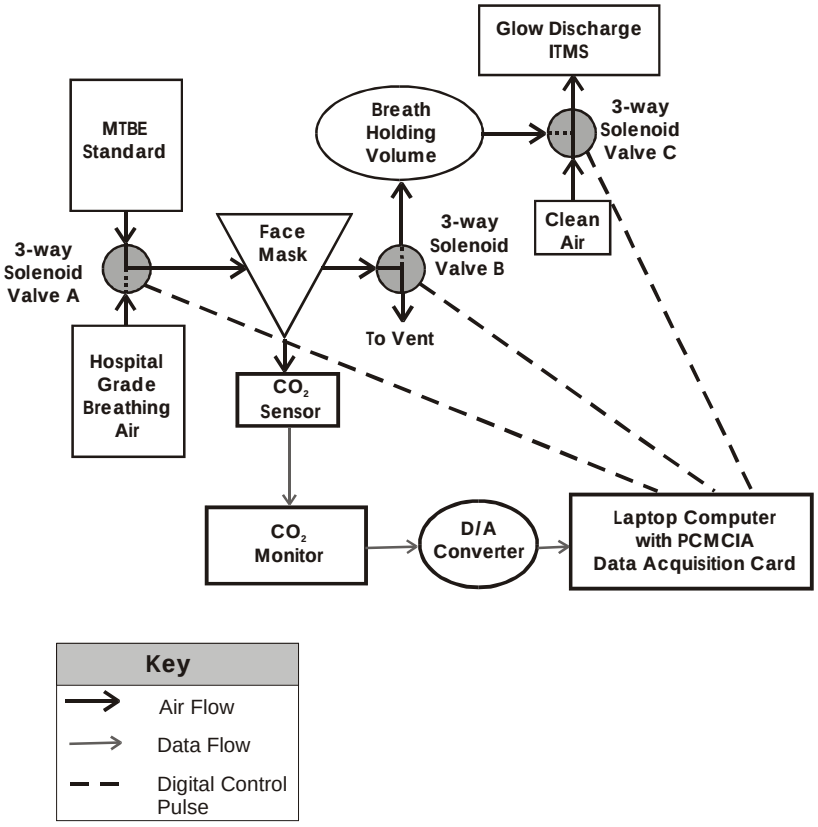

Figure 4-3. Diagram of instrumentation to measure target contaminant breath concentration continuously in real time during inhalation exposure to the contaminant. Schematic shows initial configuration of Valves A, B, and C at time t = 0 min. The breath inlet (breath holding volume) and breath analyzer are shown in greater detail in Figure 4-2.

Table 4-2. Mass spectral parent and product ions used to monitor inhalation exposure to MTBE-d_{12} and DBCM.

Compound	MW	Parent Ion	Product Ion
MTBE-d_{12}	100	82	46, 50
DBCM	208	129	—
TBA-d_{10}	84	82	62

To calibrate the real-time breath analyzer in the laboratory, gas standards containing MTBE-d_{12} and DBCM were prepared in high-pressure aluminum gas cylinders. As indicated earlier, the concentrations of the standards were confirmed by taking samples from the cylinders in evacuated 6-L stainless steel canisters, which were analyzed by a modified U.S. EPA Method TO-14.[40] The gas chromatograph/flame ionization detector/quadrupole mass spectrometer (GC/FID/MS) system, in turn, was calibrated by analyzing aliquots taken from a gravimetrically-prepared standard. Calibration of the breath analyzer itself was accomplished by connecting a gas cylinder containing the standards to the glow discharge source inlet and measuring the resultant ion signals of the target ions at the known concentrations. The instrument was calibrated each day before experiments began.

Instrument calibrations were checked by first dynamically diluting a standard six-component cylinder (LL17298), which contains the target chemicals MTBE, MTBE-d_{12}, and DBCM, as well as chloroform, benzene, and 2-methyl-2-propanol. From the known concentrations of these compounds in the cylinder (8-13 ppbv), we were able to generate average response factors. The cylinders that were taken to the field for the exposure study were similarly diluted, and the concentrations determined by GC/MS, based on the measured concentrations of the components in standard cylinder LL17298. These values were checked, in turn, by applying the generated response factor for MTBE to the measurement of the concentration of MTBE in a Scott Specialty Gas MTBE Certified Standard. Our measured values of 54.25 ppbv and 52.67 ppbv divided by the dilution factor of 0.0495 gave an estimated cylinder concentration of 1,080 ppbv versus a certified value of 1,030 ppbv. This agreement is regarded as satisfactory and allowed us to use the generated response factors to determine concentrations of the target compounds in the exposure experiments.

A CO_2 monitor (Pryon Model No. SC-300), equipped with an external infrared CO_2 sensor, is used to continuously monitor the subjects' breath CO_2 levels. The monitor is equipped with a digital-to-analog converter (Pryon Model No. D2A-8000) with a 10 ms update response time for the CO_2 waveform. The CO_2 analog waveform ranges from 0 – 10 Vdc, corresponding to 0 – 7% CO_2 (automatically corrected for water vapor). The CO_2 analog waveform data are collected using a PCMCIA data acquisition card (National Instruments Model No. DAQCard 1200), installed in a Dell laptop computer. A graphical user interface (GUI) was developed and tested, using LabView (Ver. 4.0.1) software, to acquire the relevant CO_2 data, count the number of breaths the subject takes of the exposure standard or clean air, and control the three valves that regulate the flow of the exposure standard to the subject, and subject's breath into the analyzer.

The CO_2 analog waveform data, and the digital pulses that control the solenoid valves, are also collected in a comma-delimited format for later analysis using spreadsheet software.

To measure the uptake of the target compounds during an inhalation exposure episode, the exposure time is divided into a number of discrete exposure/clean air cycles. For a single cycle, at t = 0 min, Valve A (in Figure 4-3) is set to allow flow of the MTBE-d_{12}/DBCM standard for a fixed period (say, 3 min) from the supply cylinder to the subject via the face mask, while Valve B vents the exhaled flow from the face mask and Valve C permits the analyzer to sample clean air. At t = 3n min, where n = 1, 2, 3, …, Valve A switches to allow flow of clean air to the face mask, in order to clear the tracheal dead volume of residual MTBE-d_{12}/DBCM standard, while Valve B continues to vent the flow of the first exhalation from the face mask and Valve C continues to direct clean air into the breath analyzer. After completing two full breath inhalation/exhalation cycles of clean air, Valve B switches to allow the next two clean air breath exhalations into the 200-mL tube of the breath interface device ("holding volume") and Valve C switches so that the analyzer samples from the breath holding volume for a set period of time (say 0.5 min). After the fourth clean air breath is exhaled into the holding volume, Valve B switches to prevent flow from the face mask into the holding volume, and Valve A is switched so that the subject resumes breathing the MTBE-d_{12}/DBCM standard to begin another exposure/-clean air cycle. After the analyzer has sampled from the breath holding volume, Valve C is switched so that the analyzer resumes sampling clean air.

The GUI that allows the operator to input parameters that control the inhalation exposure scenario, and then monitor subject exposure parameters and CO_2 levels in breath, is shown in Figure 4-4. Before conducting an inhalation exposure experiment, the user inputs the following parameters.

a) Inhale/Exhale Cycle Time – the time(s) it takes the subject, when at rest and breathing normally, to inhale and exhale one breath. This value is used to provide the subject with on-screen "Inhale" and "Exhale" prompts in order to help him/her maintain steady, symmetrical breathing rates. The value to be used for this time is determined empirically by monitoring the subject's breath CO_2 levels prior to the start of the inhalation exposure experiment, and measuring the time (distance) between two adjacent peaks.

b) MTBE Exposure Time – the time period(s), in a single exposure/clean air cycle, during which the subject breathes the MTBE-d_{12}/DBCM standard.

c) GDMS Sampling Time – the time period(s), in a single exposure/clean air cycle, during which the breath analyzer (GDMS) samples from the breath holding volume.

d) CO2 Max Threshold – the level of the analog signal (Vdc) above which an inhalation is counted.

e) CO2 Min Threshold – the level of the analog signal (Vdc) below which an exhalation is counted.

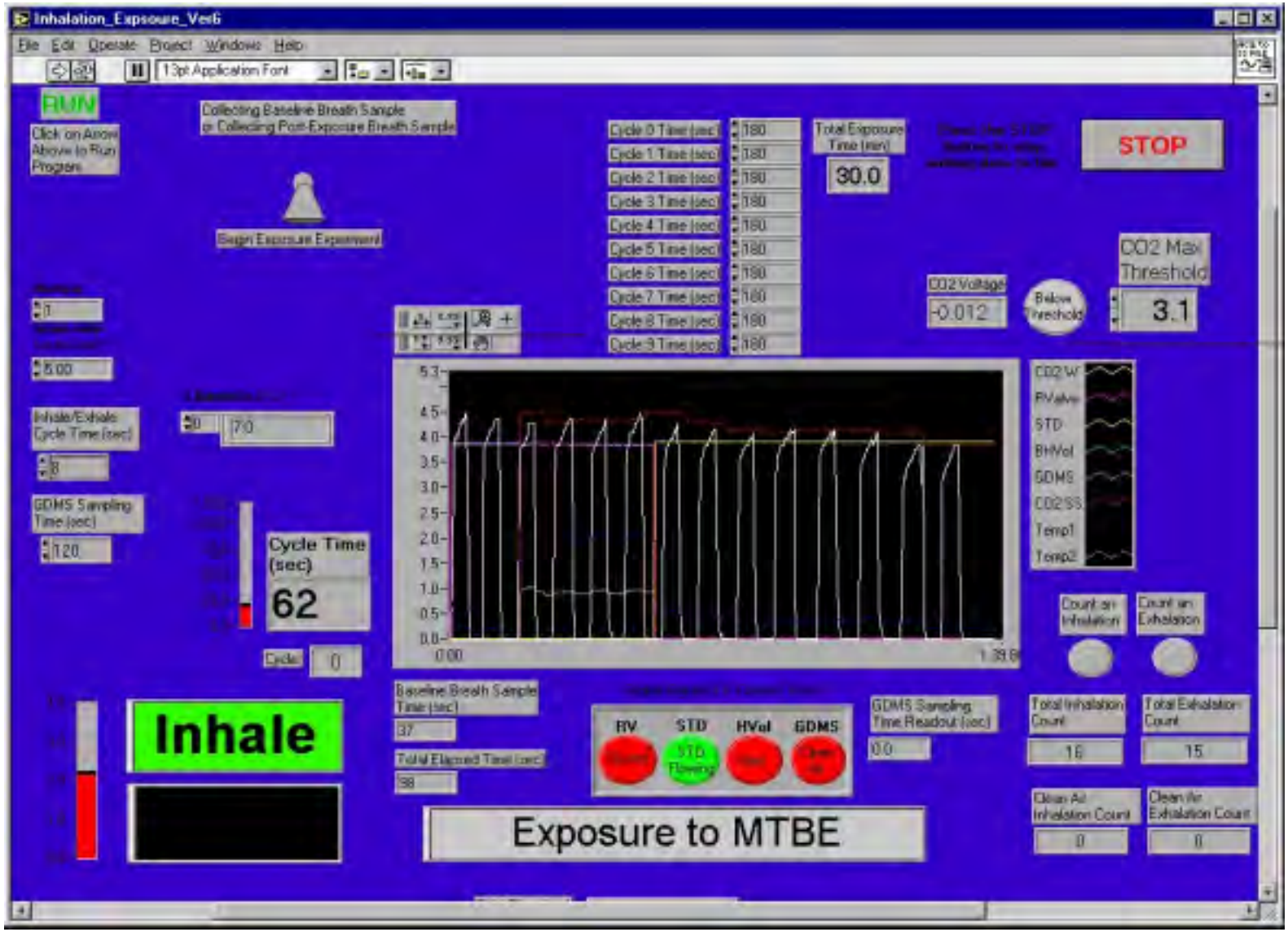

Figure 4-4. Graphical user interface for inhalation exposure data acquisition and control program.

Once these parameters have been entered, the operator launches the program. The program prompts the operator to provide a filename for the file in which the data will be stored. Once the filename is entered, the program then generates the digital pulses that control the solenoid valves, monitor the CO_2 analog data, and count the subject's breaths. The CO_2 analog data and the digital pulses are collected, plotted on the screen, evaluated, and written to a file continuously while the program runs. Timing and logic determine when digital pulses are sent to control the flow of the MTBE-d_{12}/DBCM standard, the flow of the subject's breath into the breath holding volume, and the flow of the sample from the breath holding volume into the breath analyzer. These cycle processes occur automatically without the need for operator attention, and are summarized in greater detail, along with their high and low signal states, in Table 4-3.

While the inhalation exposure software program runs, the operator can monitor the progress of the experiment by observing the additional program output windows in Figure 4-4.

f) Subject Exhale/Inhale Coach – this window prompts the subject to inhale or exhale in order to help the subject maintain a steady, symmetrical breathing rhythm. For example, if the Inhale/Exhale Cycle Time [see a) above] is 8 sec, then the subject will be "coached" to inhale for 4 sec, exhale for 4 sec, inhale for 4 sec, etc.

g) Exposure Source – this window indicates whether the subject is being exposed to the MTBE-d_{12}/DCBM standard or the clean air supply.

h) Analog Input – this window plots all of the data being collected and stored in a file, e.g., breath CO_2 levels, cycle process digital pulse states, etc.

i) Digital Signal LED cluster – this window shows a series of 8 LEDs. Currently, only 3 of the 8 LEDs are being used to indicate control of cycle processes. The LED states and the cycle processes they represent are fully described in Table 4-2.

Table 4-3. Cycle processes and their high and low signal states that are controlled by the inhalation exposure software program.

Cycle Process	High State (+5 Vdc)	Low State (0 Vdc)
Inhalation source	Subject breathes MTBE-d_{12}/DBCM standard (STD LED = red)	Subject breathes clean air (STD LED = green)
Exhalation source	Subject's breath is vented to waste (HVol LED = red)	Subject's breath is collected in the breath holding volume (HVol LED = green)
GDMS analyzer source	GDMS analyzer samples from the breath volume (GDMS LED = red)	GDMS analyzer samples from hospital grade clean air (GDMS LED = green)

j) Breath Count – total number of breaths taken by the subject since the inhalation exposure experiment started. A breath is defined as one inhalation and one exhalation cycle.

k) Clean Air Breath Count – total number of clean air breaths taken by the subject for a single exposure/clean air cycle. This value is reset to zero at the end of the GDMS sampling portion of each exposure/clean air cycle.

Initial tests conducted with the system indicated that the breath holding volume (~95 mL) was too small and the sample was being depleted during the sampling period, since the breath levels following exposure first increased, then abruptly leveled off at much lower levels than expected. To address this issue, as well as several others that were indicated by the initial tests, several modifications were made to the inhalation exposure system and software program. The changes included:

(1) The breath holding volume was increased to 500 mL by incorporating a long Teflon sampling loop (754 cm long, 0.92 cm i.d.). The front end of the sampling loop was fitted with a 2-way solenoid relief valve such that, when the breath analyzer is permitted to sample from the back end of the sampling loop, clean air is able to enter the front of the loop through the relief valve, thus avoiding the formation of a vacuum and keeping the sampling loop at atmospheric pressure. The on/off switching of this relief valve was also placed under automatic control by including its operation in the software program. This change resulted in stable breath analyzer sampling periods of up to 4 min from the loop when filled with 500 mL of a test gas standard (1,1,1-trichloroethane).

(2) The time period (s) for which the subject is exposed to the exposure standard was modified to be user-selectable so that it could be varied for each individual exposure cycle. Each exposure cycle consists of three separate periods: the time for which the subject is exposed to the standard; the time it takes the subject to complete 4 clean-air breaths; and the time for which the breath analyzer samples from the breath holding volume. Each exposure experiment can consist of 10-15 cycles. Prior to this change, the exposure standard time period was user-selectable, but its duration could not be varied between cycles. The time periods that are selected for each cycle for a given inhalation exposure experiment are attached to the end of the comma-delimited data file when the experiment is halted (i.e., the STOP button is clicked).

(3) The subject's inhalations and exhalations were programmed to be counted separately, beginning with the subject's first inhalation. This change was made to ensure that a single breath is more accurately defined as an inhalation followed by an exhalation. The clean air and exposure-standard inhalations and exhalations are shown in four separate program output windows (see lower right hand corner of Figure 4-4). The clean air inhalations and exhalations are each reset to zero at the beginning of a new exposure cycle.

(4) A switch was included in the program to allow the user to choose between running the inhalation exposure experiment, and stopping the experiment and sampling from the

breath holding volume for any period of time. This change was made to allow the collection of a baseline breath sample prior to running the inhalation exposure experiment, and to allow continuous monitoring of the breath during the post-exposure decay period. In addition to the inhalation exposure phase, the CO_2 levels are monitored throughout the pre- and post-exposure phases, and are all recorded in a single comma-delimited file.

The effects of all these changes were tested by conducting several inhalation exposure measurements, using a standard of 50 ppbv (270 µg/m^3) 1,1,1-trichloroethane in humidified air. The results obtained are shown in Figure 4-5, where the experimentally generated data points are compared with a curve generated from the linear compartment model, using values for the residence times for 1,1,1-trichloroethane reported earlier by Wallace et al.[45]

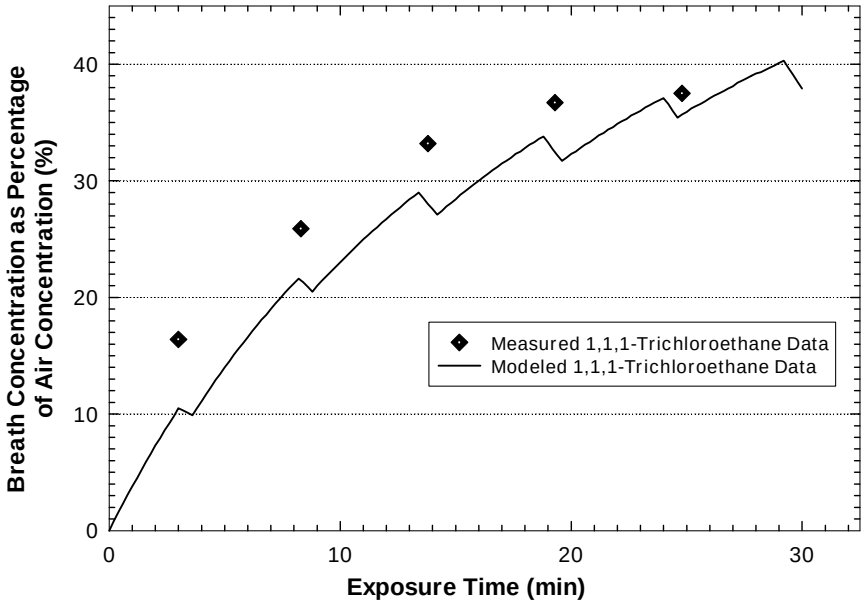

Figure 4-5. Modeled (solid line) and measured (asterisks) inhalation uptake of 1,1,1-trichloroethane in exhaled breath of a subject exposed to 50 ppbv (270 µg/m^3) 1,1,1-trichloroethane in air. For the curve calculated from the linear compartment model, we assumed $f = 0.87$; $\tau_1 = 9.0$ min; $\tau_2 = 41$ min; and $\tau_3 = 288$ min (from Wallace et al.[45])

Blood and Urine

All blood and urine samples were analyzed by a purge-and-trap method using Tenax (Supelco Inc., PA) as an adsorbent (0.25 g in each trap) and zero grade helium (Air Products and Chemicals Inc., PA) as the purge gas.[15,16,46] The sampling traps were conditioned by continuous flushing with zero-grade nitrogen while being heated at 270°C for 3 hours. The trap was

repacked with fresh Tenax sorbent after twenty uses. Newly packed traps were conditioned by flushing with zero-grade nitrogen while being heated at 270°C for 6 hours. To evaluate breakthrough, two traps were connected in series and each was analyzed for all of the experiments. Calibration curves were prepared using only the first trap in the series as well as the sum obtained from both traps.

Blood

Multiple blood samples were taken from each subject over several hours, using a Jelco Winged IV Catheter (Johnson & Johnson, NJ), which remained in the subject's arm for the duration of the experiment. Blood samples were drawn by a trained phlebotomist, who verified that the catheter was not causing undue discomfort or other problems while it remained in the arm.

The samples were collected into 10 mL Vacutainers® (Benton Dickson, NJ) with 20 mg of potassium oxalate and 25 mg of sodium fluoride, and stored at 4°C until analysis. A 10 mg/L disodium-EDTA solution (Baker Analyzed ACS Reagent, JT Baker, Phillipsburg, NJ) was prepared with HPLC-reagent water (JT Baker, Phillipsburg, NJ), which had non-detectable levels of MTBE. An 8-mL aliquot of the blood sample was transferred to a 250-mL gas bubbler vessel containing 100 mL of the disodium-EDTA solution. One mL of antifoaming solution (Dow Corning Antifoam® 1510-US Emulsion, Midland, MI) was added to prevent foaming. The gas bubbler containing the sample was first immersed in a water bath at 40°C for 3 minutes before purging to allow it to reach temperature equilibrium. Then, the blood-disodium-EDTA mixture was purged with helium gas at 100 mL/min for 10 minutes at 40°C to collect MTBE-d_{12} and DBCM. Next, the gas bubbler was transferred to a water bath at 90°C, allowed to come to temperature equilibrium over 4 minutes, and the sample was further purged for an additional 10 minutes at 90°C to collect TBA.

Recovery tests were run with standards (24 ng of each compound) using both EDTA mixed with 10 mL of Bacteriostatic sodium chloride injection solution 0.9% (Abbott Laboratories, IL) and EDTA mixed with blood. The blood was obtained from individuals not exposed to MTBE or DBCM. No difference in recovery between the two matrices was observed. Consequently, the EDTA-sodium chloride matrix was used to prepare spiked standards for generation of calibration curves and system evaluation rather than spiked EDTA-blood matrices.

Urine

A 200-mL urine sample was transferred to a 250 mL gas bubbler. One drop of antifoaming solution (Dow Corning Antifoam® 1510-US Emulsion, Midland, MI) was added to the 200-mL urine sample to prevent foaming during purging. The gas bubbler containing the urine sample was first immersed in a water bath at 40°C for 3 minutes before purging to allow it to reach temperature equilibrium. Then, the urine was purged with helium gas at 150 mL/min for 10 minutes at 40°C to collect MTBE-d_{12} and DBCM on the Tenax traps. Next, the gas bubbler was transferred to a water bath at 90°C, allowed to come to temperature equilibrium over

4 minutes, and the urine sample was further purged for an additional 10 minutes at 90°C to collect TBA.

GC/MS Analysis

Target compounds were analyzed and quantified using a gas chromatograph (Hewlett Packard 5890) coupled to a quadrupole mass spectrometer (Hewlett Packard 5971A Mass Selective Detector). Analytes were stripped from the Tenax trap and transferred to the GC/MS system by thermal desorption (Perkin-Elmer, Inc, Model ATD-400). A 60 m, 5% diphenyl-95% dimethyl polysiloxane capillary column (DB-5, 0.25 mm ID, 1 μm film thickness; J & W Scientific, Folsom, CA) was used.

The GC temperature conditions were: injector 250° C; oven held at 35°C for 8 min, then ramped at 10°/min to 170°C, ramped at 50°/min to 220°C, and held for 5 min. The target ions for deuterated MTBE and TBA were m/e = 82 and 68, respectively, and their retention times were 9.5 and 7.9 minutes, respectively. Ion intensity-area data were used to determine relative response factors (RRF) for the compounds on each day the instrument was operated. This was accomplished by injecting bromofluorobenzene (BFB) and ^{13}C-benzene, using amounts similar to those obtained from the purged samples.

The detection limit (DL) for each compound in the blood and urine samples was determined by estimating the standard deviation of the blank (σ_B) and the level of the analytical noise (y_B). The standard error of the regression line was used as an estimated standard deviation of the blank, and the intercept of the regression line was used as an estimate of the analytical noise. The method detection limit (MDL) were calculated from

$$y_{DL} - y_B = 3\sigma_B$$

where σ_B = standard error of the regression line; y_B = intercept of the regression line; and y_{DL} = signal level. When $y = y_{DL}$, DL has the value of x.

Questionnaire

A brief questionnaire (shown in Appendix A) was administered to each participant to assess the participant's potential exposure to MTBE and DBCM during the previous 24 hours.

Data Analysis

The data from the inhalation exposure uptake and decay of MTBE-d_{12} and DBCM were evaluated in terms of a linear multi-exponential compartment model, developed by Wallace et al.,[29] which allowed us to estimate the total ("applied") dose, the "unmetabolized mass", and the total absorbed dose in addition to the distribution and residence times of the chemicals in breath and blood corresponding to different body compartments.

Total ("Applied") Dose

The total ("applied") dose to the subject is determined from the product of the total exposure and the subject's average alveolar ventilation rate. Figure 4-6 depicts the form of the model for exposure to a constant high concentration for a time *T*. For this condition,

$$\text{Total Exposure, } E_{total} = C_{air} \cdot T \quad (\mu\text{g.min/m}^3) \tag{4-1}$$

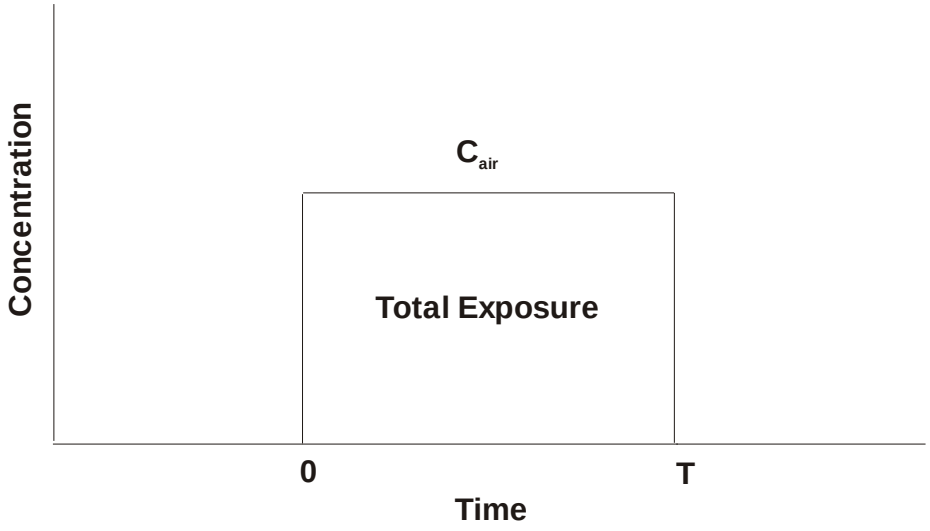

Figure 4-6. Step function exposure to a constant air concentration C_{air} for time *T*.

and Total ("Applied") Dose = (Total Exposure) · (Alveolar Ventilation Rate) (μg)

$$= E_{total} \cdot AVR$$
$$= (C_{air}T) \cdot AVR \tag{4-2}$$

where C_{air} = constant exposure concentration (μg/m^3); T = total duration of exposure to the constant concentration C_{air} (min); and AVR = alveolar ventilation concentration (L/min).

Total Absorbed Dose

The total absorbed (internal) dose to the subject is defined as the amount of the chemical that passes through an absorption barrier or exchange boundary. It is given by the difference between the total ("applied") dose and the "unmetabolized mass."[47,48,49]

"Unmetabolized Mass"

The "unmetabolized mass" is the total mass of the chemical that leaves the body via exhalation.[47] It is obtained by multiplying the sum of the areas under the exhaled breath uptake and decay curves by the alveolar ventilation rate.

For the situation in which an exposure at relatively high concentrations is followed immediately by exposure to clean air, as depicted in Figure 4-7, it follows that the value of the alveolar breath concentration at the beginning of the exposure to clean air, i.e., at time $t = T$, is largely determined by the previous exposure. Then, in the case of a single compartment, for the uptake phase:

$$C_{alv} = fC_{air}\left(1 - e^{-t/\tau}\right) \qquad \text{for } t \le \text{T} \qquad (4\text{-}3)$$

and for the elimination phase, we have:

$$C_{alv} = fC_{air}\left(1 - e^{-T/\tau}\right)e^{-(t-T)/\tau} \qquad \text{for } t \ge \text{T} \qquad (4\text{-}4)$$

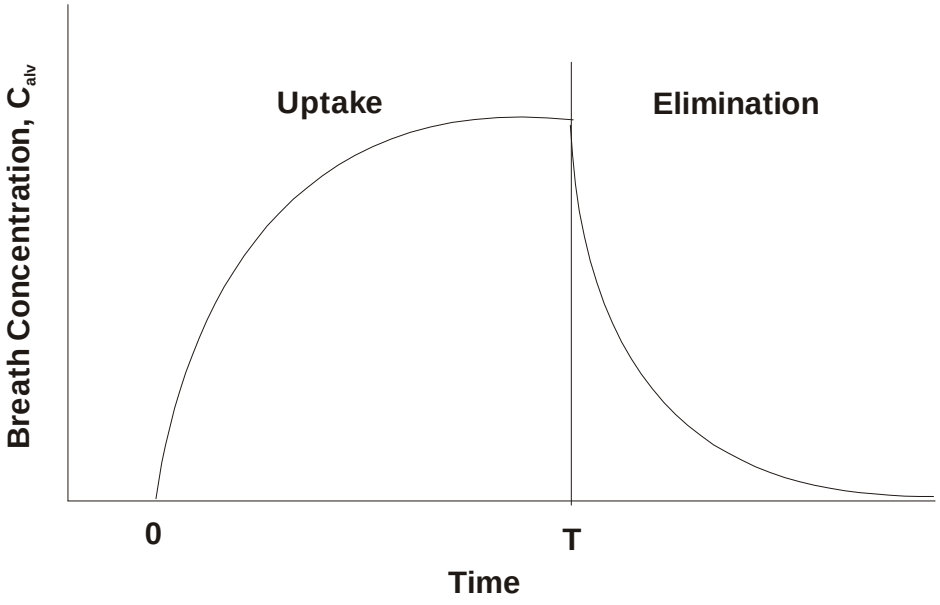

Figure 4-7. Plot showing rapid increase in alveolar breath concentration C_{alv} as a result of step function exposure to a constant air concentration C_{air}, followed by a rapid decrease in breath concentration as a result of exposure to clean air.

where C_{alv} = exhaled alveolar breath concentration of the component; f = fraction of inhaled breath concentration exhaled at equilibrium; and t = time from the start of the exposure.

Then, the area under the uptake curve, AUC_{uptake}, is given by:

$$
\begin{aligned}
AUC_{uptake} &= \int_0^T C_{alv}\,dt \\
&= fC_{air}\int_0^T \left(1-e^{-t/\tau}\right)dt \\
&= fC_{air}\left[t-\left(-\tau e^{-t/\tau}\right)\right]_0^T \\
&= fC_{air}\left[T-\tau+\tau e^{-T/\tau}\right] \\
&= fC_{air}T - fC_{air}\tau\left(1-e^{-T/\tau}\right)
\end{aligned}
\qquad (4\text{-}5)
$$

Since Equation (4-5) contains the parameter *f* that is to be determined from this area, AUC_{uptake} is estimated instead by integrating under the exponentially increasing curve used to model the data, i.e., $y = a(1 - e^{-bx})$, using the trapezoidal rule in SigmaPlot (Version 5.0, SPSS, Chicago, IL).

The area under the decay curve, AUC_{decay}, is given by:

$$
\begin{aligned}
AUC_{decay} &= \int_T^\infty C_{alv}\,dt \\
&= \int_T^\infty fC_{air}\left(1-e^{-T/\tau}\right)e^{-(t-T)/\tau}\,dt \\
&= fC_{air}\,e^{T/\tau}\left(1-e^{-T/\tau}\right)\int_T^\infty e^{-t/\tau}\,dt \\
&= fC_{air}\,e^{T/\tau}\left(1-e^{-T/\tau}\right)\left[-\tau e^{-t/\tau}\right]_T^\infty \\
&= -fC_{air}\,\tau\,e^{T/\tau}\left(1-e^{-T/\tau}\right)\left[0-e^{-T/\tau}\right] \\
&= fC_{air}\tau\left(1-e^{-T/\tau}\right)
\end{aligned}
\qquad (4\text{-}6)
$$

(For the two-compartment case, the expression for AUC_{decay} is:

$$
AUC_{decay} = fC_{air}\,a_1\,\tau_1\left(1-e^{-T/\tau_1}\right) + fC_{air}\,a_2\,\tau_2\left(1-e^{-T/\tau_2}\right) \qquad (4\text{-}7)
$$

where the coefficient a_i is the fractional contribution of the *i*th compartment to the breath at equilibrium; and τ_I is the residence time of the chemical in the *i*th compartment.)

Since Equation (4-6) again contain the parameter *f* that has not yet been determined, we have adopted an alternative approach for the practical determination of AUC_{decay}. For the post-exposure decay period, the model may be written in the form:[29]

$$
C_{alv} = fC_{air} + \sum a_i e^{-t/\tau_i} \qquad (4\text{-}8)
$$

where, now, $t = 0$ denotes the time exposure ends; and fC_{air}, the fraction of the inhaled air concentration of the chemical that is exhaled, is zero during elimination. It follows then, in general, that:[49]

$$\begin{aligned} AUC_{decay} &= \int_0^\infty C_{alv}\,dt \\ &= \int_0^\infty \sum a_i e^{-t/\tau_i}\,dt \\ &= -\sum a_i\,\tau_i \left[e^{-t/\tau_i}\right]_0^\infty \\ &= -\sum a_i\,\tau_i\,(0-1) \\ &= \sum a_i\,\tau_i \end{aligned} \tag{4-9}$$

Thus, AUC_{decay} may be estimated in practice from the best-fit parameters obtained from the exponentially decreasing multi-compartment curve used to model the decay data, i.e., $y = \Sigma a_i e^{-bx}$.

For the one-compartment case, the total area under the uptake and decay curves, AUC_{total}, follows from Equations (4-5) and (4-6):

$$AUC_{total} = fC_{air} \cdot T \tag{4-10}$$

and the "unmetabolized mass" (i.e., total amount (μg) exhaled during uptake and decay) is given by:

$$\begin{aligned} \text{"Unmetabolized Mass"} &= AUC_{total} \cdot AVR \\ &= fC_{air} \cdot T \cdot AVR \end{aligned} \tag{4-11}$$

The fraction f of the chemical exhaled unchanged at equilibrium may be estimated from the ratio of the "unmetabolized mass" to the total ("applied") dose, i.e.,

$$f\cdot(\text{Total Dose}) = \text{"Unmetabolized Mass"} \tag{4-12}$$

It follows then that the total absorbed dose (μg) may be estimated from:

$$\begin{aligned} \text{Total Absorbed Dose} &= (\text{Total Dose}) - (\text{"Unmetabolized Mass"}) \\ &= C_{air} \cdot T \cdot AVR - fC_{air} \cdot T \cdot AVR \\ &= C_{air} \cdot T \cdot AVR(1-f) \end{aligned} \tag{4-13}$$

The fraction of the chemical absorbed (i.e., "relative uptake"[16]) is calculated from:

$$\text{Fraction of Chemical Absorbed} = \frac{E_{total} - AUC_{total}}{E_{total}} = 1 - f \tag{4-14}$$

Finally, the fraction of the chemical eliminated through expiration after exposure (i.e., "exhaled post-exposure"[16]) is estimated from:

$$\begin{aligned}\text{Fraction of Chemical Exhaled Post - Exposure} &= \frac{\text{Amount Exhaled Post - Exposure}}{\text{Total Absorbed Dose}} \\ &= \frac{AUC_{decay} \cdot AVR}{\text{Total Absorbed Dose}}\end{aligned} \tag{4-15}$$

Empirical Modeling of Uptake and Decay Breath and Blood Concentrations

The linear multicompartment model has the following solution:[29]

$$C = fC_{air} \sum a_i \left(1 - e^{-t/\tau_i}\right) \tag{4-16}$$

where: C = exhaled breath or blood concentration of the component; a_i = capacity of the i^{th} compartment at equilibrium ($\Sigma a_i = 1$); t = time from the onset of exposure; and τ_i = residence time of the chemical in the i^{th} compartment. Pleil et al.[50] have pointed out that, when Equation (4-16) is applied to blood data, the term for the exposure concentration C_{air} is, in fact, a composite parameter that includes an adjustment for the effective transfer of the gas phase to the blood (the blood/breath partition coefficient P) that accounts for Henry's Law.

The fraction f of the compound exhaled unchanged at equilibrium, i.e., $t = \infty$, follows from Equation (4-16) as:

$$f = \frac{C_{t=\infty}}{C_{air} \sum a_i} \tag{4-17}$$

During the post-exposure decay phase, the concentration declines exponentially:

$$C = fC_{air} + \sum a_i e^{-t/\tau_i} \tag{4-18}$$

where, now, t is measured from the time exposure ends. In the experiment conducted here, the air concentration C_{air} was set to zero, i.e., $fC_{air} = 0$. In Equation (4-18), the first exponential term (compartment) is generally associated with blood, the second with "highly perfused tissues," the third with "moderately perfused tissues," and the fourth with "poorly perfused tissues." For a broad range of VOCs, it has been found that the residence times for these compartments are roughly similar, namely, 3-11 min for the first compartment, 0.4-1.6 h for the second, 3-8 h for the third, and several days for the fourth compartment.[45] For the exposure times used in the present study, we

apply a two-compartment decay model to evaluate the contributions to the breath levels during the decay period.

The residence time is defined as the time it takes for the compound to decay to $1/e$ of its initial concentration in the compartment, assuming all other compartments are at zero concentration. The biological half-life $t_{½}$ of the compound in the body is related to the residence time τ through the relation:

$$\tau = t_{½}/\ln 2 \qquad (4\text{-}17)$$

All of the parameters are determined empirically using the Marquardt-Levenberg (nonlinear regression) algorithm in SigmaPlot (Version 5.0, SPSS, Chicago, IL), which minimizes the differences in the sum of squares between the assumed model and the experimental data. This analysis provides values for the a, τ, and $t_{½}$ terms.

The model may also be used to estimate the concentration of the component in the blood at any time during the elimination phase from the relation: $C_{blood} = C_{alv}.P$, where C_{blood} is the concentration of the component in the blood and P is the blood/breath partition coefficient. The modeled breath values at $t = 0$, i.e., when exposure ceases, together with the relation for C_{blood}, provide an estimate of the maximum blood levels of the component attributable to the exposure.

Quality Control

Four types of samples were collected in this study: exhaled breath, room air, blood, and urine. Exhaled breath samples were collected and analyzed simultaneously using the real-time breath analyzer; whole-air samples were collected in stainless steel canisters and analyzed by cryogenic preconcentration followed by GC/MS, using a modified U.S. EPA Method TO-14.[40] Blood and urine samples were analyzed using purge and trap procedures.[15,16,46] For each of these analyses, calibration curves were first prepared from at least four standards. The curves are checked on a daily basis, using a standard prepared separately from the calibration standard. The tune settings on the respective analytical mass spectrometers were verified daily. Holding times for the air samples were less than one week. Laboratory blanks were analyzed on a regular basis by the respective laboratories. Reproducibility was estimated from duplicate analyses. The respective instrument minimum detection limits were determined from multipoint calibrations.

Exhaled Breath and Whole Air

The 3DQ ion trap mass calibration was established and checked each day, using routine operating procedures and internal 3DQ software designed for that purpose. Specific 3DQ operating parameters and diagnostic checks were also evaluated daily.

Calibration of response of the real-time breath analyzer to the target breath components was performed, as described earlier (cf. Chapter 4, Breath Measurements), using gas standards prepared in cylinders. Samples of the cylinder contents were collected in canisters and analyzed

using GC/MS. The concentrations of the MTBE, MTBE-d_{12}, DBCM, *tert*-butyl alcohol (TBA), and benzene in the canister samples were determined using a dynamic dilution of a gravimetrically-prepared in-house standard (Battelle standard LL-17298). This calibration mixture contains MTBE, MTBE-d_{12}, DBCM, trichloromethane, TBA, and benzene prepared at ppbv levels in nitrogen. Table 4-4 lists the target compounds and their concentrations in the standard. These concentrations were derived from a knowledge of the original amount injected and the pressure of the cylinder. The MTBE concentration was validated by analyzing a certified reference gas (Scott Specialty Gas), which was also dynamically diluted under the same conditions as the calibration standards.

Table 4-4. Battelle standard containing the target compounds, trichloromethane, and benzene in nitrogen.

Compound	Concentration (ppbv)
MTBE	9.31
MTBE-d_{12}	8.17
DBCM	12.9
Trichloromethane, $CHCl_3$	14.0
TBA	11.6
Benzene, C_6H_6	12.5

The accuracy of the Battelle standard LL-17298 was assessed, in turn, by analyzing standard LL-17305 and the MTBE certified reference gas. Using the automated GC/MS system described earlier, the resulting peak areas were used to quantify the target compounds in the MTBE reference gas and Battelle standard. Then, the concentrations of MTBE, trichloro-methane, and benzene in the standards were calculated from the peak areas using the average response factor (concentration/average peak area) obtained from Battelle standard LL-17298. Table 4-5 compares the certified and measured concentrations for the Battelle in-house and

Table 4-5. Comparison of measured and certified concentrations of MTBE in certified reference standard, and chloroform and benzene in NIST SRM 1804a.

Compound	Certified Concentration (ppbv)	Measured Concentration* (ppbv)	% Difference
MTBE	51.0[a]	53.5 ± 1.1	4.9
Chloroform	16.9[b]	16.6 ± 0.6	1.8
Benzene	14.8[b]	15.6 ± 0.8	5.4

[a] Certified reference gas (Scott Specialty Gas).
[b] With respect to Battelle LL-17298 standard.

certified standards. These results indicate that the values obtained for the concentrations of MTBE, chloroform, and benzene in Battelle standard Ll-17298 are reliable.

The calibration standard for the real-time breath analyzer was prepared in-house by static dilution in a 15.7 L cylinder. To prepare the standard, an intermediate standard consisting of 360 µL of pure DBCM (Aldrich, 98% purity) was diluted to a final 2.0 mL volume with methanol. To prepare the 3DQ calibration standard, 1.2 µL of the intermediate standard and 0.6 µL of pure MTBE-d_{12} (C/D/N Isotopes, >99.8% atom % D) were injected into a 15.7 L cylinder through a heated syringe injection port attached to the cylinder. The cylinder then was pressurized to 1,000 psig using medical grade breathing air (Praxair). A canister sample was collected and analyzed in duplicate using the modified EPA TO-14 method and the automated GC/MSD/FID system described earlier. The measured FID peak areas were used to quantify the MTBE-d_{12} and DBCM in the sample. Then, as before, the concentrations of MTBE-d_{12} and DBCM in the canister were calculated from the FID peak area using the average response factor (concentration/average peak area) obtained from Battelle standard LL-17298. The concentration of MTBE-d_{12} estimated in this way was 119.7 ppbv compared with the concentration injected, viz., 100.0 ppbv, which represents a 19.7 percent difference. The concentration of DBCM estimated in this way was 53.9 ppbv compared with the concentration injected, viz., 57.3 ppbv, which represents a 5.9 percent difference. The good agreement obtained between the measured and injected concentrations validates the accuracy of the spiking method, which has been used extensively in our laboratory.[28] The concentrations of MTBE-d_{12} and DBCM injected into the cylinder (100.0 and 57.3 ppbv, respectively) and the average MTBE-d_{12} and DBCM peak areas obtained for the canister sample were used to quantify the concentrations of MTBE-d_{12} and DBCM in the breath sample data acquired continuously with the real-time breath analyzer. As noted earlier, we were unable to calibrate the breath analyzer for the target compound TBA because of its apparent adsorption onto the inner surfaces of the ion trap. Consequently, the cylinders were only used to calibrate the instrument for MTBE-d_{12} and DBCM.

Blood and Urine

Quality control measures undertaken for the collection and analysis of the blood and urine samples included the following:

- All glassware used was first cleaned with 10% HCl and rinsed with de-ionized water, then baked at 300°C for 12 h before use.
- Soon after collection, all blood and urine samples were stored in a cold room at 4°C until analysis.
- Before purging a sample, helium gas was sparged through the entire system for 5 minutes to remove any MTBE contamination. In addition, to avoid DBCM contamination, the Tygon tubing connecting the purge vessel to the trap was replaced between samples.
- The operation and performance status of the GC/MS system was checked daily by analyzing 50 ng of BFB (bromofluorobenzene) and 31.6 ng of ^{13}C-benzene standards.

- Blank traps were checked for contamination by GC/MS before use in the purge-and-trap analysis. These blank traps were analyzed with each set of samples to ensure that neither the traps nor the analytical system were contaminated.
- External QC standards were prepared on Tenax traps by directly injecting the BFB/-benzene standard into a flash evaporator and flushing the vapors onto the trap with zero-grade nitrogen. The QC standards were analyzed after every sixth sample to verify the stability of the GC/MS response.

Chapter 5
Results

A number of practical difficulties were experienced while conducting the inhalation exposure experiments at EOHSI. The bathroom in which the experiments were carried out was unventilated and had no temperature control. Heat emitted by the electronics of the real-time breath analyzer and ancillary equipment during the day, along with the heat from the bodies of the operators and the subjects in the room, caused the mass scale of the breath analyzer to drift in an unexpected and unpredictable fashion, necessitating frequent and time consuming recalibrations and additional operational checks. Additionally, serious problems were subsequently encountered at EOHSI in the analyses of the blood and urine samples for the target compounds. As a result, all of the blood and urine data presented here are regarded as suspect and must be viewed with caution.

A total of seven subjects participated in the inhalation exposure experiments. Before each experiment, the subject was fitted with a venous catheter and a face mask which was connected to two gas cylinders such that the subject breathed either hospital-grade air from one of the cylinders or air containing a mixture of 2,217 $\mu g/m^3$ (542 ppbv) MTBE-d_{12} and 728 $\mu g/m^3$ (85.6 ppbv) DBCM from the second cylinder. Pre-exposure blood, breath, and urine samples were collected, followed by the exposure (uptake) period, which was of ~30 minutes duration. During the exposure period, we made an effort to collect paired blood and discrete breath samples using various time sequences. A typical sequence was: t = -5 to 0 min (pre-exposure baseline sample), then 3, 5, 20 (duplicate blood draw), and 29 min. At the start of the elimination period (at t = ~30 min), the subject's breathing tube was automatically switched to the second cylinder so that he/she breathed only hospital-grade air. Breath measurements were taken for an additional ~30 min (until t = ~60 min). After a rest period of about 25 min, an additional 5-min breath sample was taken and all monitoring terminated at t = ~90 min. In three cases, a third continuous breath sample was taken, 15 minutes later in two of these cases, and 20 minutes later in the third case. During each elimination period, three or four blood samples were taken, typically at 35, 45, and ending at either 60 or 90 min. Table 5-1 provides a summary of the collection times for the breath and blood samples in the MTBE-d_{12}/DBCM inhalation exposure experiments.

Exhaled Breath Data

All of the LabView CO_2-in-breath and valve switching data were converted to spreadsheet format; the CO_2 waveforms were converted into $\%CO_2$ based on the calibration of the CO_2 monitor with a 5% CO_2 gravimetric standard.

Table 5-1. Summary of blood and breath sample collection times (min) in each exposure experiment.

	Subject IF02		Subject IM03		Subject IM04		Subject IM05		Subject IM08		Subject IF06		Subject IM01	
MTBE-d_{12}/DBCM Conc ($\mu g/m^3$)	**2,217/728**		**2,217/728**		**2,217/728**		**2,217/728**		**2,217/728**		**2,217/728**		**2,217/728**	
Activity	**Breath**	**Blood**	**Breath**	**Blood**	**Breath**	**Blood**	**Breath**	**Blood**	**Breath**	**Blood**	**Breath**	**Blood**	**Breath**	**Blood**
Pre-Exposure	-3.3	-5	-2.5	-5	-2.9	-5	-2.3	-5	-4.3	-5	-4.6	-5	-5.0	-5
Exposure Start	0.00		0.00		0.00		0.00		0.00		0.00		0.00	
Exposure uptake monitored[a]	2.45	—	2.42	—	2.34	—	2.55	—	1.74	—	1.43	3.0	1.56	—
Exposure uptake monitored[a]	5.99	5.0	6.49	5.0	5.85	5.0[b]	5.92	5.0[b]	4.45	5.0	3.84	5.0	4.24	5.0
Exposure uptake monitored[a]	9.35	—	10.34	—	9.14	—	9.58	—	7.24	10.0	6.18	—	6.48	—
Exposure uptake monitored[a]	12.98	15.0	14.12	15.0	12.72	15.0	13.16	15.0	12.90	—	9.57	—	11.24	—
Exposure uptake monitored[a]	17.52	—	18.95	—	17.18	—	17.69	—	18.71	—	15.97	—	17.64	15.0
Exposure uptake monitored[a]	22.61	—	23.85	—	21.64	—	22.96	—	25.28	28.0	22.60	20.0	24.72	—
Exposure uptake monitored[a]	27.69	29.0	29.59	29.0	27.27	29.0	—	29.0	32.98	—	31.12	29.0	—	29.0
Exposure uptake monitored[a]	33.32	—	36.45	—	—	—	—	—	35.69	—	33.53	—	—	32.0[b]
1st Continuous Post-Exposure Start[c]	33.51		36.68		33.49		33.85		35.84		33.75		34.23	
Exposure decay monitored	↓	35.0	↓	32.0	↓	35.0	↓	35.0[*]	↓	35.0[b]	↓	35.0[b]	↓	35.0
Exposure decay monitored	↓	45.0	↓	—	↓	45.0	↓	45.0	↓	—	↓	45.0	↓	45.0
Exposure decay monitored	↓	60.0	↓	60.0	↓	60.0	↓	60.0	↓	60.0	↓	60.0	↓	60.0
1st Continuous Post-Exposure Stop	62.11		60.45		64.42		64.27		66.10		64.34		60.08	
2nd Continuous Post-Exposure Start[c]	83.67		85.32		90.42		89.15		91.10		89.34		90.08	
Exposure decay monitored	↓	90.0	↓	90.0	↓	90.0	↓	—	↓	90.0	↓	—	↓	90.0
2nd Continuous Post-Exposure Stop	91.21		90.54		94.73		94.74		96.45		94.17		95.18	

	Subject IF02		Subject IM03		Subject IM04		Subject IM05		Subject IM08		Subject IF06		Subject IM01	
MTBE-d_{12}/DBCM Conc (µg/m^3)	**2,217/728**		**2,217/728**		**2,217/728**		**2,217/728**		**2,217/728**		**2,217/728**		**2,217/728**	
Activity	**Breath**	**Blood**	**Breath**	**Blood**	**Breath**	**Blood**	**Breath**	**Blood**	**Breath**	**Blood**	**Breath**	**Blood**	**Breath**	**Blood**
3rd Continuous Post-Exposure Start[c]			115.7		118.7				125.1					
			↓		↓				↓					
3rd Continuous Post-Exposure Stop			120.7		124.0				130.2					

[a] Breath concentration during exposure period monitored at discrete times by temporarily interrupting the exposure to collect the exhaled breath sample. See text for details.

[b] Duplicate samples taken.

[c] Breath concentration during post-exposure (decay) period monitored continuously in real time (indicated by vertical arrow).

For the inhalation exposure experiments, CO_2-in-breath and valve switching data workup included:

- Preparing a summary of clean air breaths taken by, and respiration rates of, each subject during each exposure cycle
- Determining the following parameters for the three distinct periods of each inhalation experiment; i.e., pre-exposure (baseline) breath, exposure period, and post-exposure (decay) period:
    - number of inhalations
    - number of exhalations
    - total volume of air expired
    - average tidal volume
    - respiration rate

Figures 5-1 to 5-7 show the continuous uptake and elimination breath profiles obtained for MTBE-d_{12} and DBCM from the seven subjects. Buckley et al.[12] have pointed out that to characterize the breath uptake profile during inhalation exposure, it is necessary to temporarily interrupt the exposure to the target pollutants with inhalations of pure air. Otherwise, the collected breath samples would be masked, especially in the early stages of the exposure, by the residual high levels of the target chemicals from the supply source. To circumvent this problem, at fixed times during the exposure period (see Table 5-1), the biofeedback system shown in Figure 4-3 was used to automatically switch the subject from the MTBE-d_{12}/DBCM supply source to inhalation of pure air from a separate cylinder for 1 – 2 min while continuing to exhale into the breath monitoring system. At the end of each of these brief breath sampling periods, the supply was switched back to the MTBE-d_{12}/DBCM supply source and the exposure uptake resumed until the next measurement sequence. Figures 5-8 to 5-14 show the uptake and elimination breath profiles obtained after averaging the measurements taken during the uptake phase using this procedure.

Breath and Blood Data

As indicated by the summary of experiments in Table 5-1, paired blood data for MTBE-d_{12} were obtained for the uptake and elimination periods for all of the subjects who provided breath samples. Measurable concentrations for TBA in blood were also obtained for three of the seven subjects who participated, but blood levels for DBCM were below the limits of detection in all cases.

Figures 5-15 to 5-21 show the uptake and elimination concentrations of MTBE-d_{12} in the breath and the blood for all seven subjects. The results obtained for TBA in blood are also included in Figures 5-19 to 5-21 for Subjects IM08, IF06, and IM01, respectively.

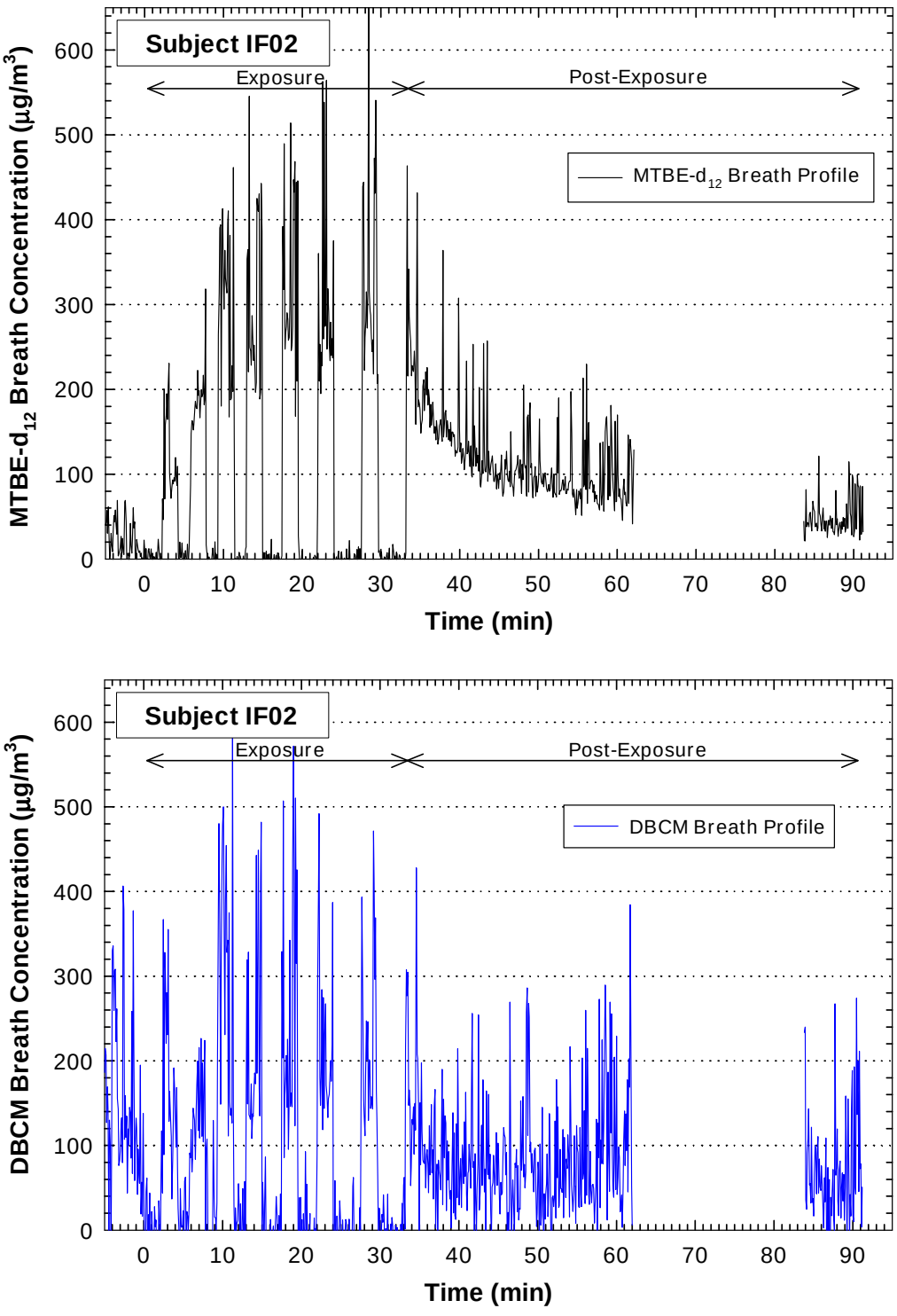

Figure 5-1. Continuous uptake and decay profiles of MTBE-d_{12} (upper plot) and DBCM (lower plot) in breath for female Subject IF02 exposed to 2,217 µg/m^3 (542 ppbv) of MTBE-d_{12} and 728 µg/m^3 (85.6 ppbv) of DBCM in air for 29.3 minutes (effective exposure period).

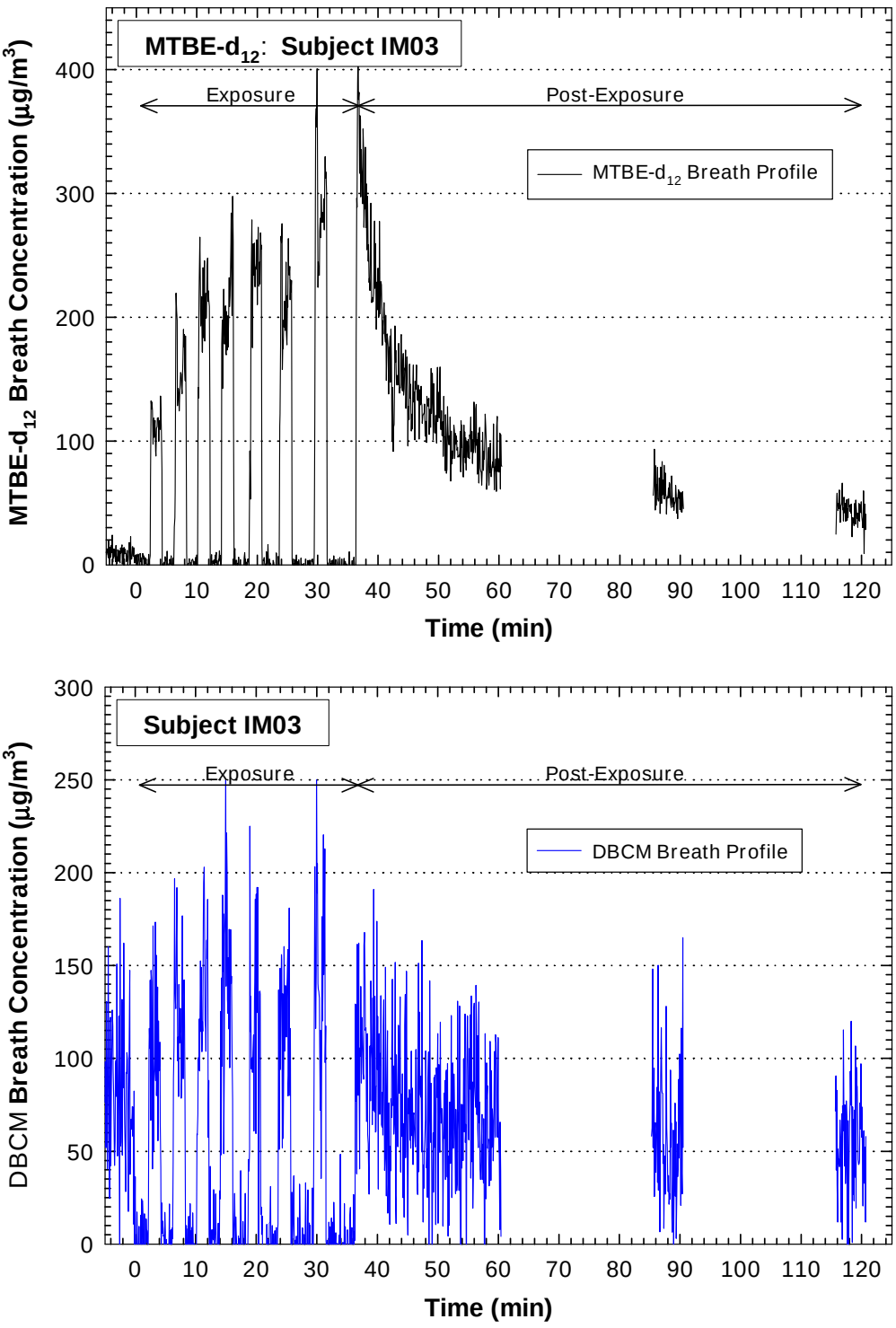

Figure 5-2. Continuous uptake and decay profiles of MTBE-d_{12} (upper plot) and DBCM (lower plot) in breath for male Subject IM03 exposed to 2,217 µg/m^3 (542 ppbv) of MTBE-d_{12} and 728 µg/m^3 (85.6 ppbv) of DBCM in air for 30.6 minutes (effective exposure period).

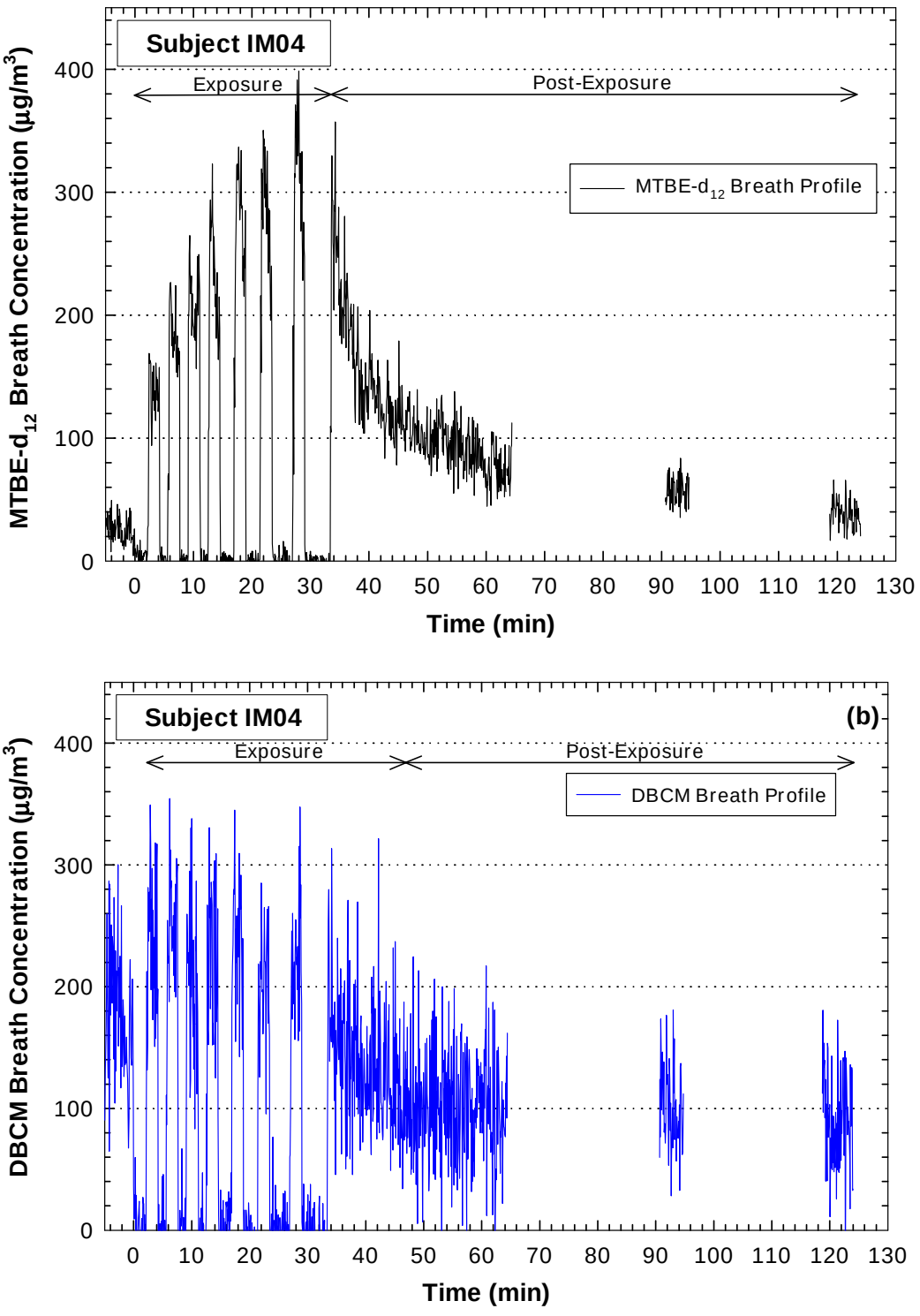

Figure 5-3. Continuous uptake and decay profiles of MTBE-d_{12} (upper plot) and DBCM (lower plot) in breath for male Subject IM04 exposed to 2,217 µg/m^3 (542 ppbv) of MTBE-d_{12} and 728 µg/m^3 (85.6 ppbv) of DBCM in air for 30.3 minutes (effective exposure period).

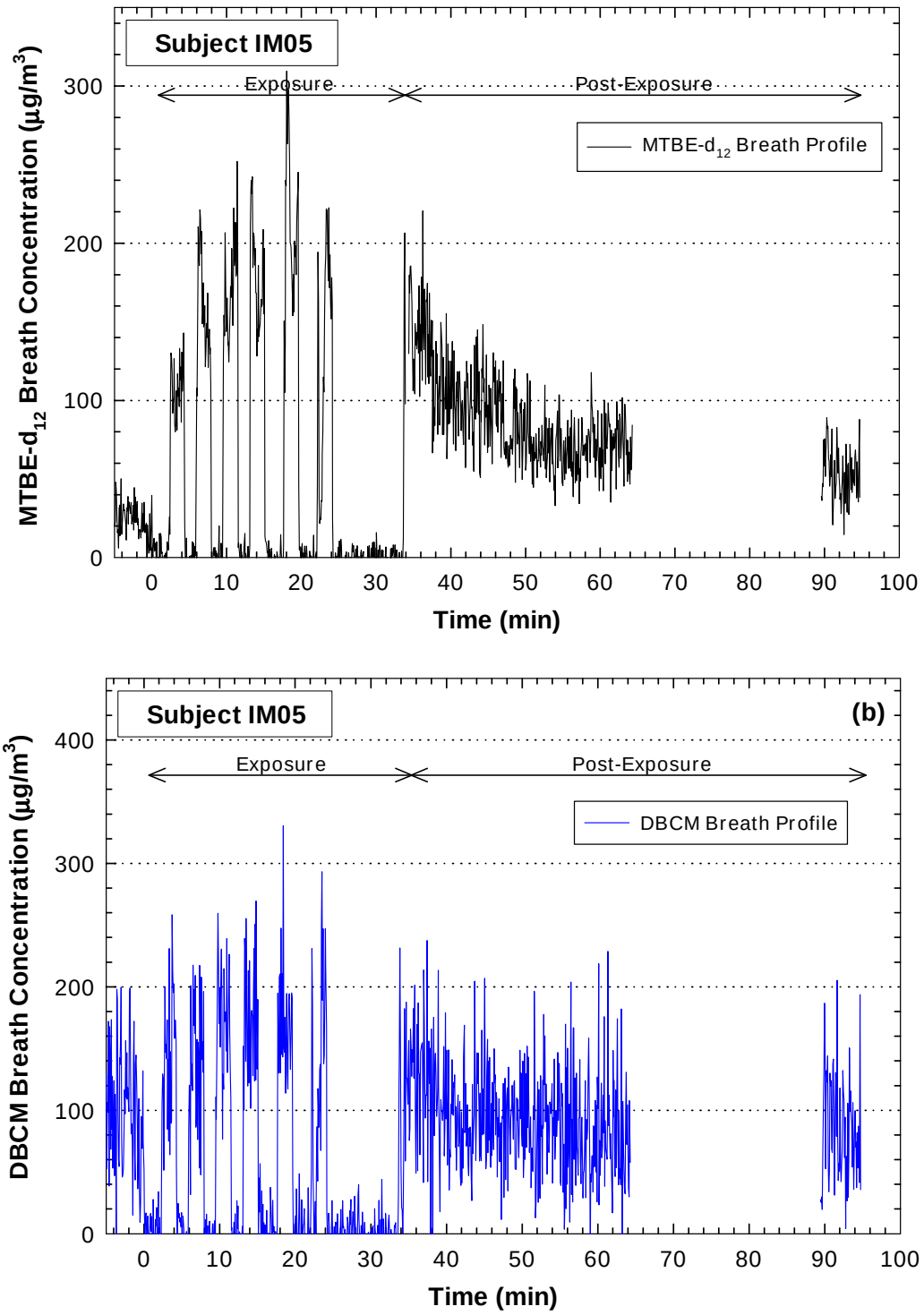

Figure 5-4. Continuous uptake and decay profiles of MTBE-d_{12} (upper plot) and DBCM (lower plot) in breath for male Subject IM05 exposed to 2,217 µg/m^3 (542 ppbv) of MTBE-d_{12} and 728 µg/m^3 (85.6 ppbv) of DBCM in air for 30.6 minutes (effective exposure period).

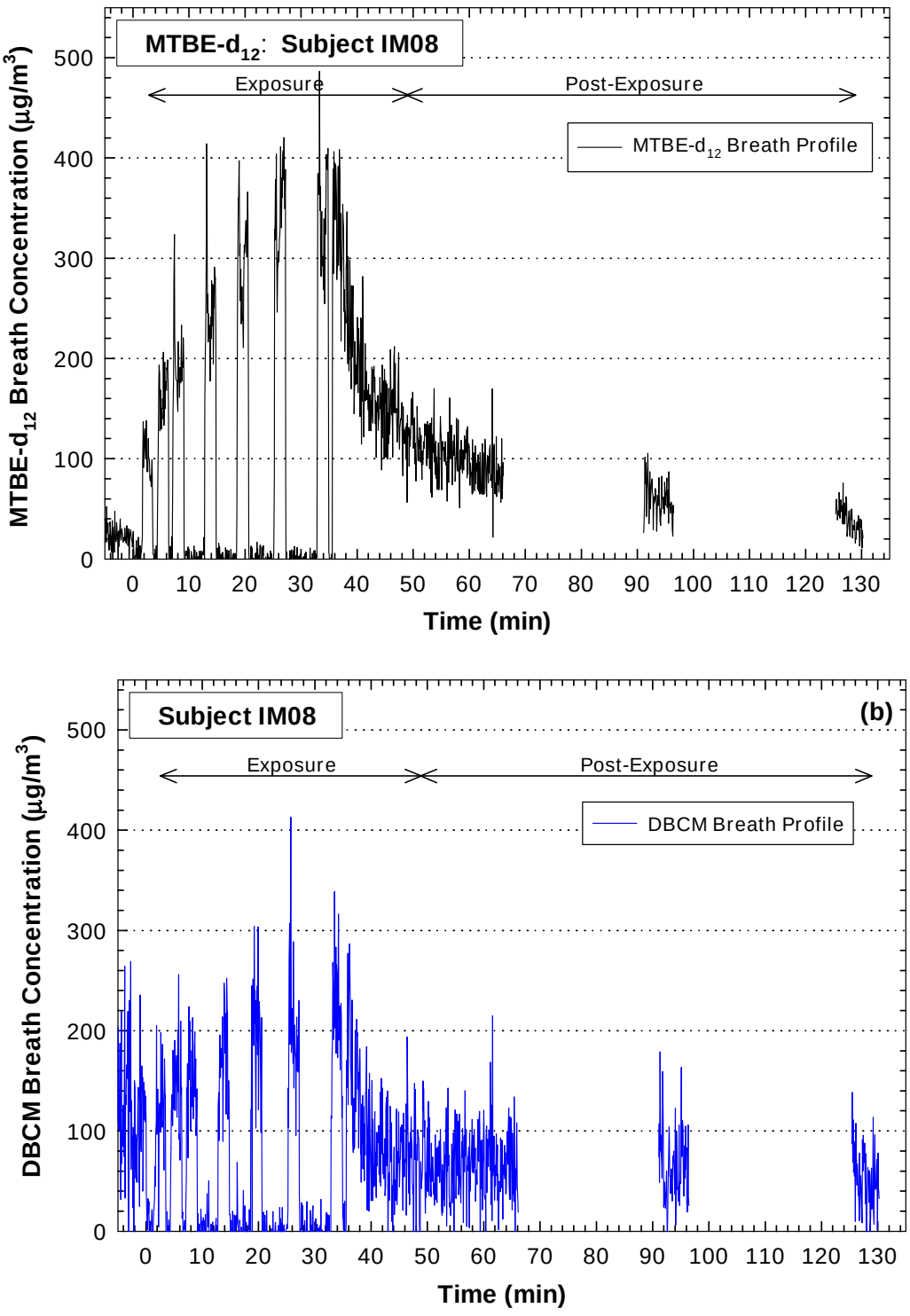

Figure 5-5. Continuous uptake and decay profiles of MTBE-d_{12} (upper plot) and DBCM (lower plot) in breath for male Subject IM08 exposed to 2,217 µg/m^3 (542 ppbv) of MTBE-d_{12} and 728 µg/m^3 (85.6 ppbv) of DBCM in air for 30.6 minutes (effective exposure period).

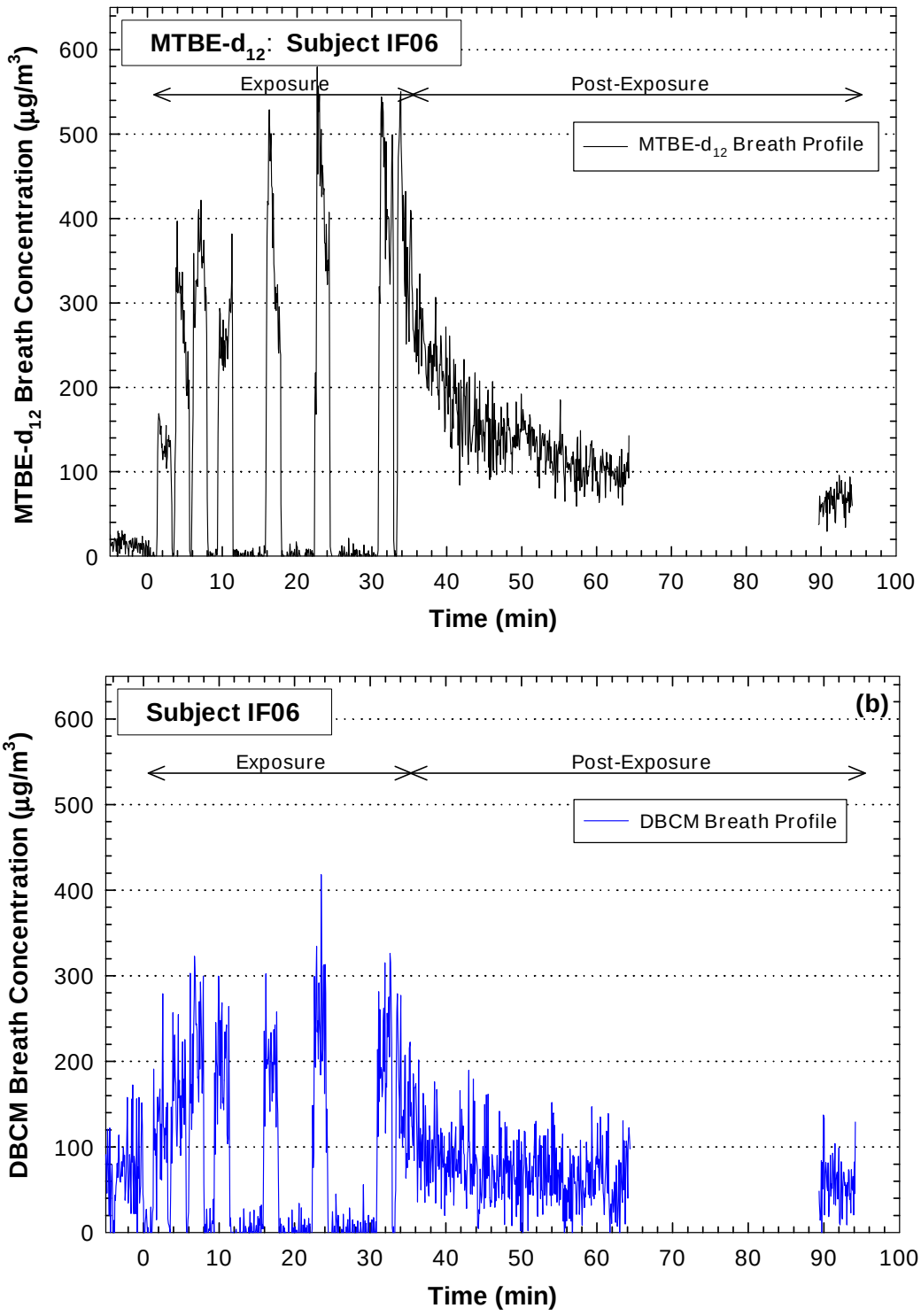

Figure 5-6. Continuous uptake and decay profiles of MTBE-d_{12} (upper plot) and DBCM (lower plot) in breath for female Subject IF06 exposed to 2,217 µg/m^3 (542 ppbv) of MTBE-d_{12} and 728 µg/m^3 (85.6 ppbv) of DBCM in air for 30.7 minutes (effective exposure period).

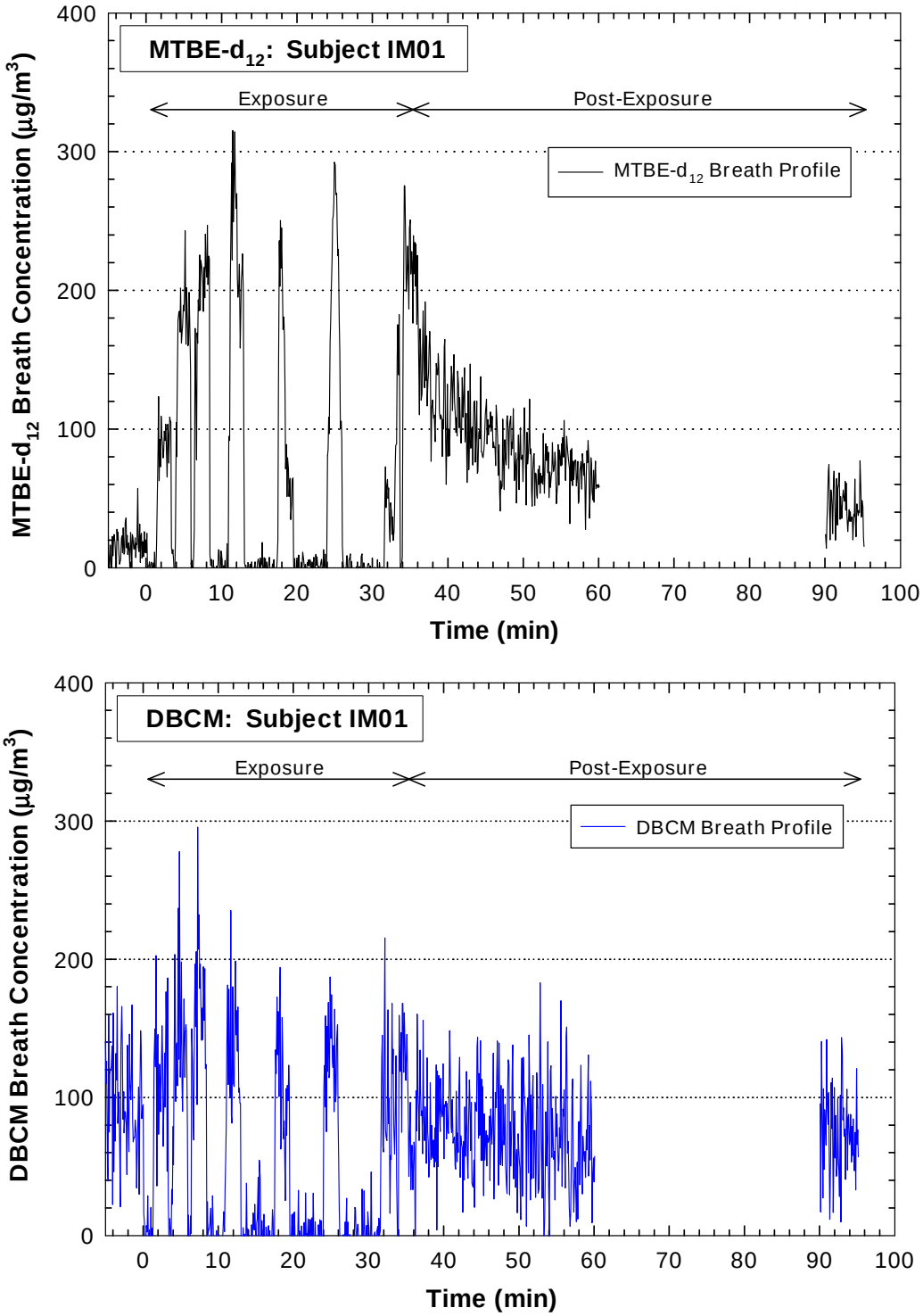

Figure 5-7. Continuous uptake and decay profiles of MTBE-d_{12} (upper plot) and DBCM (lower plot) in breath for male Subject IM01 exposed to 2,217 µg/m^3 (542 ppbv) of MTBE-d_{12} and 728 µg/m^3 (85.6 ppbv) of DBCM in air for 30.5 minutes (effective exposure period).

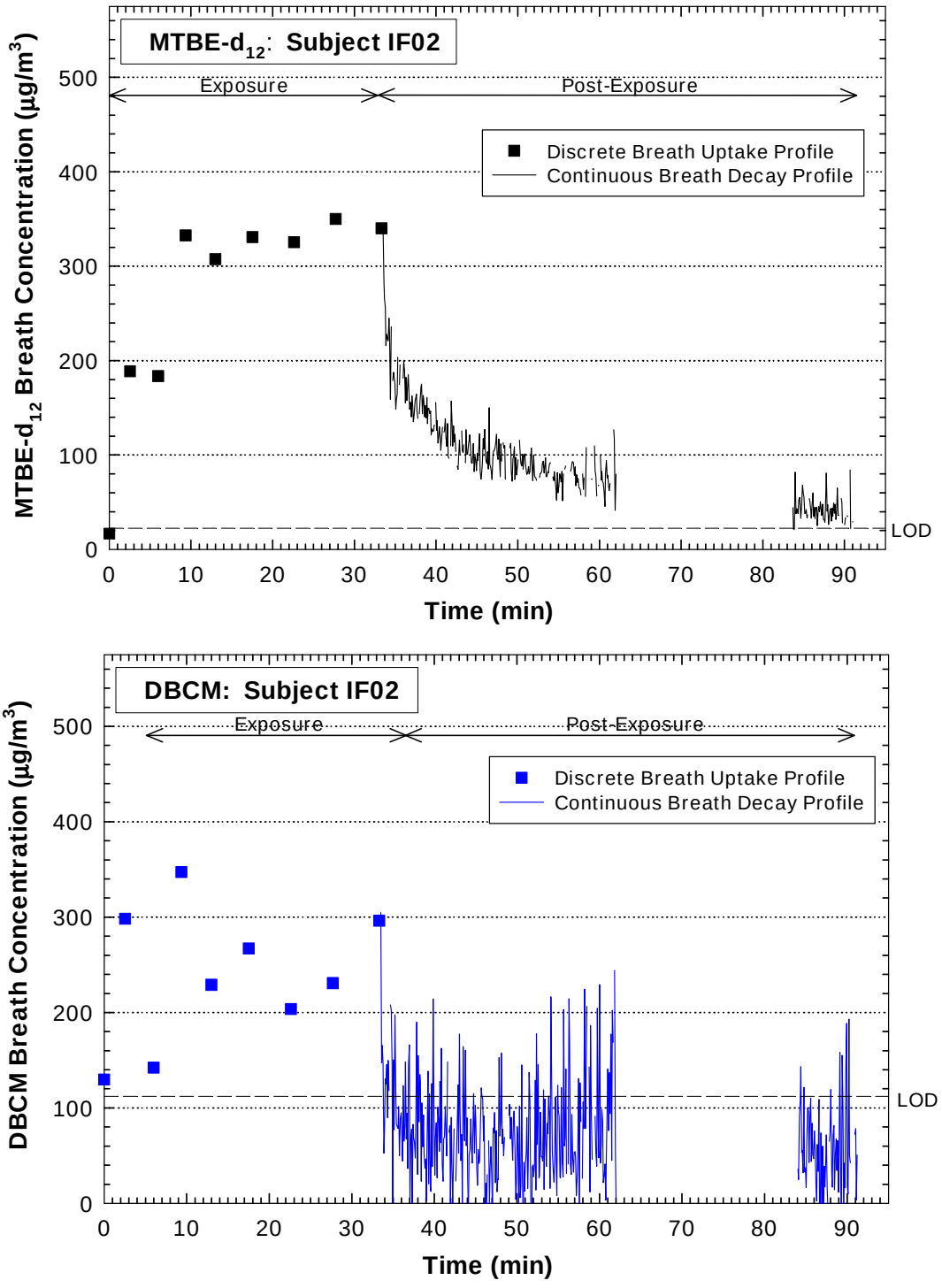

Figure 5-8. Discrete uptake and continuous decay profiles of MTBE-d_{12} (upper plot) and DBCM (lower plot) in breath for female Subject IF02 exposed to 2,217 µg/m^3 (542 ppbv) of MTBE-d_{12} and 728 µg/m^3 (85.6 ppbv) of DBCM in air for 29.3 minutes (effective exposure period). LOD designates limit of detection for target compound.

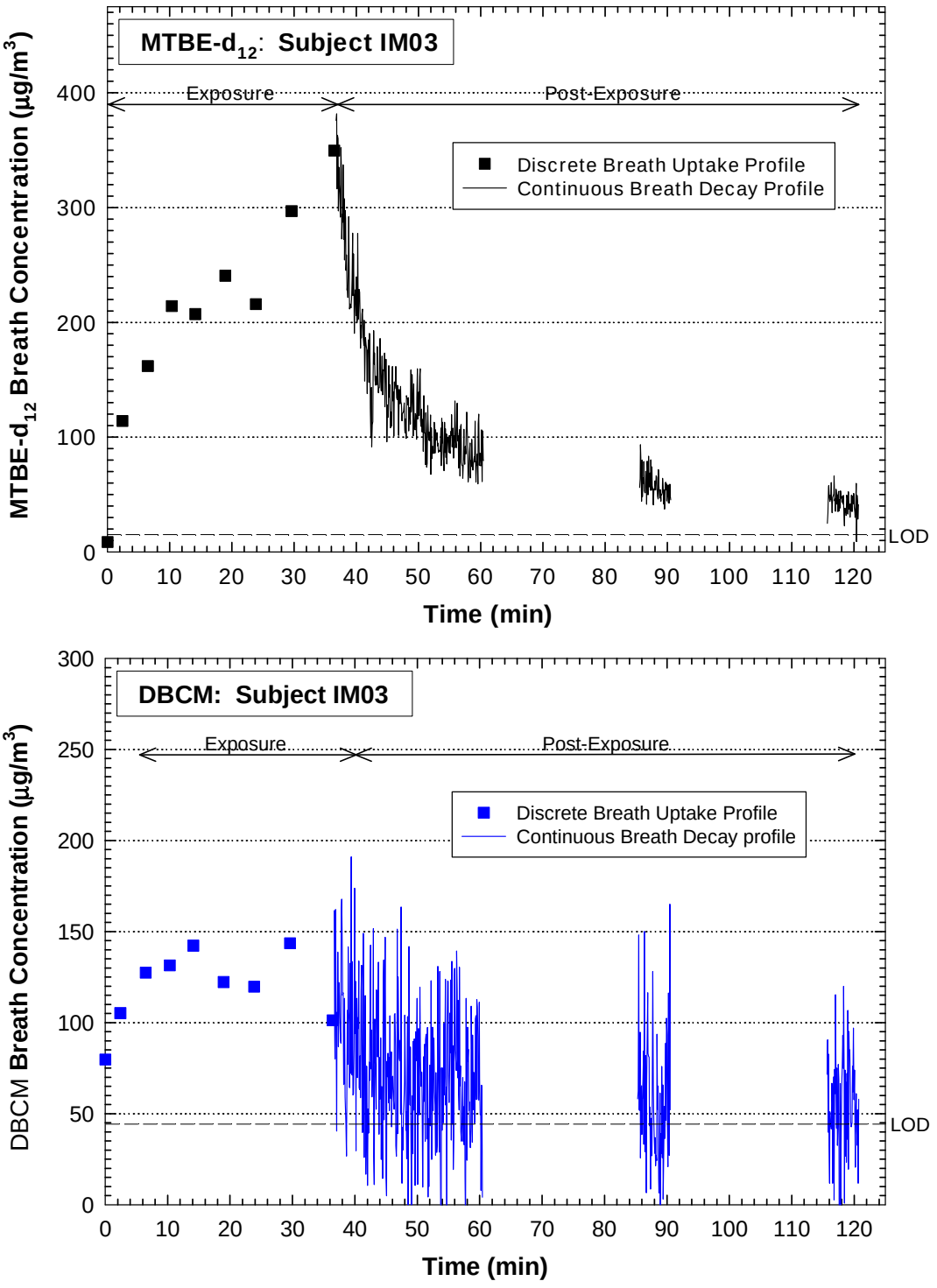

Figure 5-9. Discrete uptake and continuous decay profiles of MTBE-d_{12} (upper plot) and DBCM (lower plot) in breath for male Subject IM03 exposed to 2,217 µg/m^3 (542 ppbv) of MTBE-d_{12} and 728 µg/m^3 (85.6 ppbv) of DBCM in air for 30.6 minutes (effective exposure period). LOD designates limit of detection for target compound.

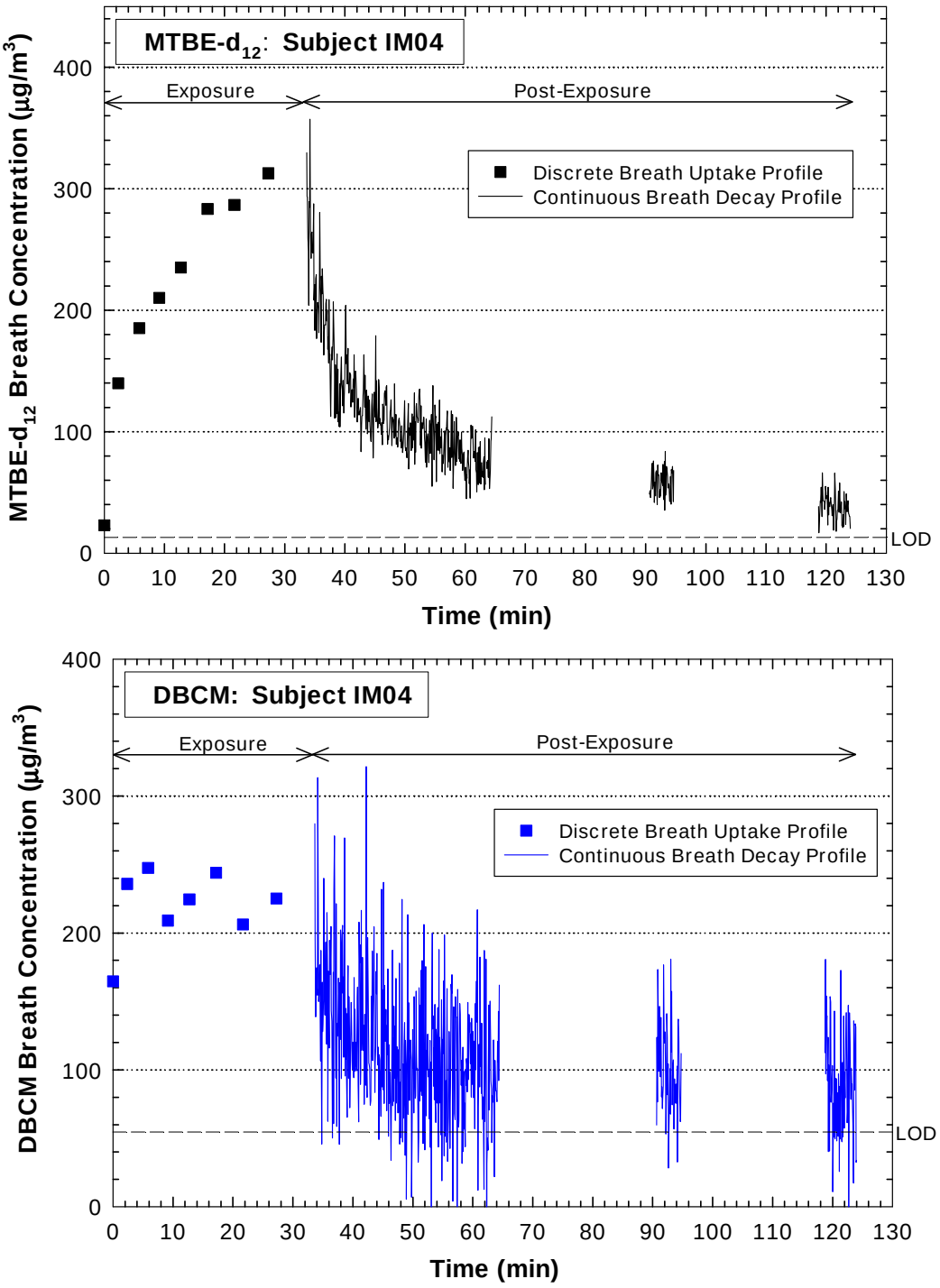

Figure 5-10. Discrete uptake and continuous decay profiles of MTBE-d_{12} (upper plot) and DBCM (lower plot) in breath for male Subject IM04 exposed to 2,217 µg/m^3 (542 ppbv) of MTBE-d_{12} and 728 µg/m^3 (85.6 ppbv) of DBCM in air for 30.3 minutes (effective exposure period). LOD designates limit of detection for target compound.

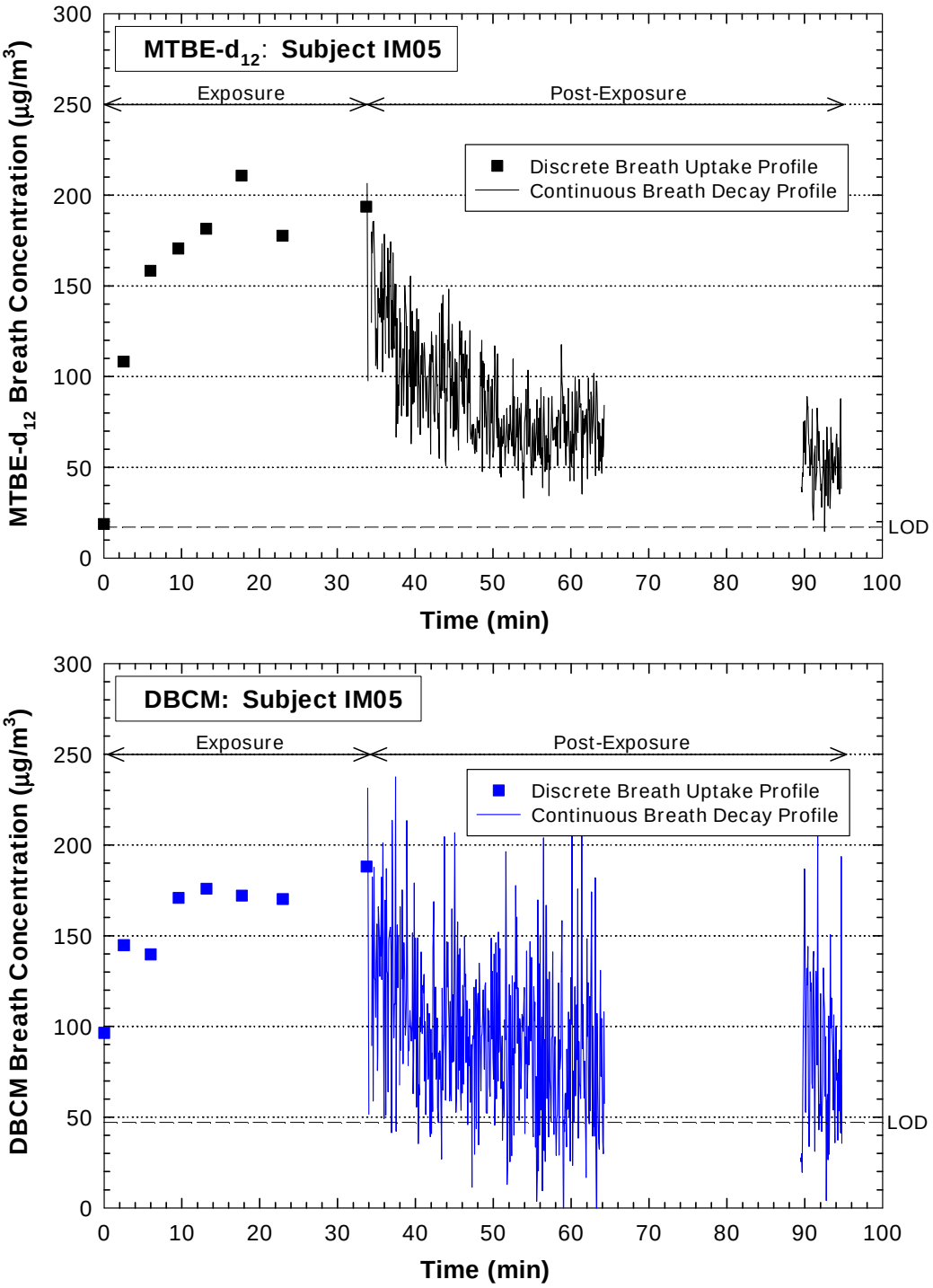

Figure 5-11. Discrete uptake and continuous decay profiles of MTBE-d_{12} (upper plot) and DBCM (lower plot) in breath for male Subject IM05 exposed to 2,217 µg/m^3 (542 ppbv) of MTBE-d_{12} and 728 µg/m^3 (85.6 ppbv) of DBCM in air for 30.6 minutes (effective exposure period). LOD designates limit of detection for target compound.

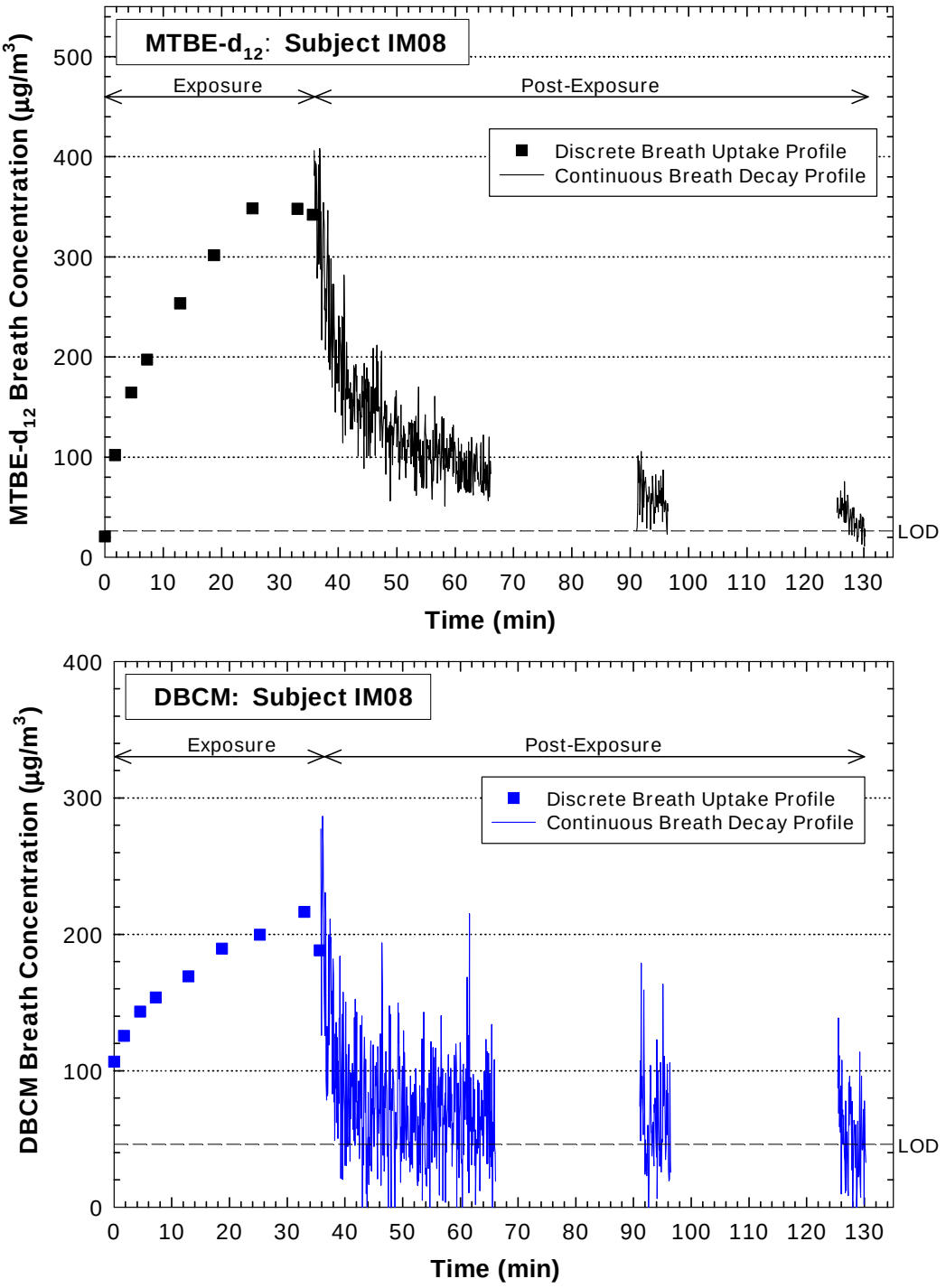

Figure 5-12. Discrete uptake and continuous decay profiles of MTBE-d_{12} (upper plot) and DBCM (lower plot) in breath for male Subject IM08 exposed to 2,217 µg/m^3 (542 ppbv) of MTBE-d_{12} and 728 µg/m^3 (85.6 ppbv) of DBCM in air for 30.6 minutes (effective exposure period). LOD designates limit of detection for target compound.

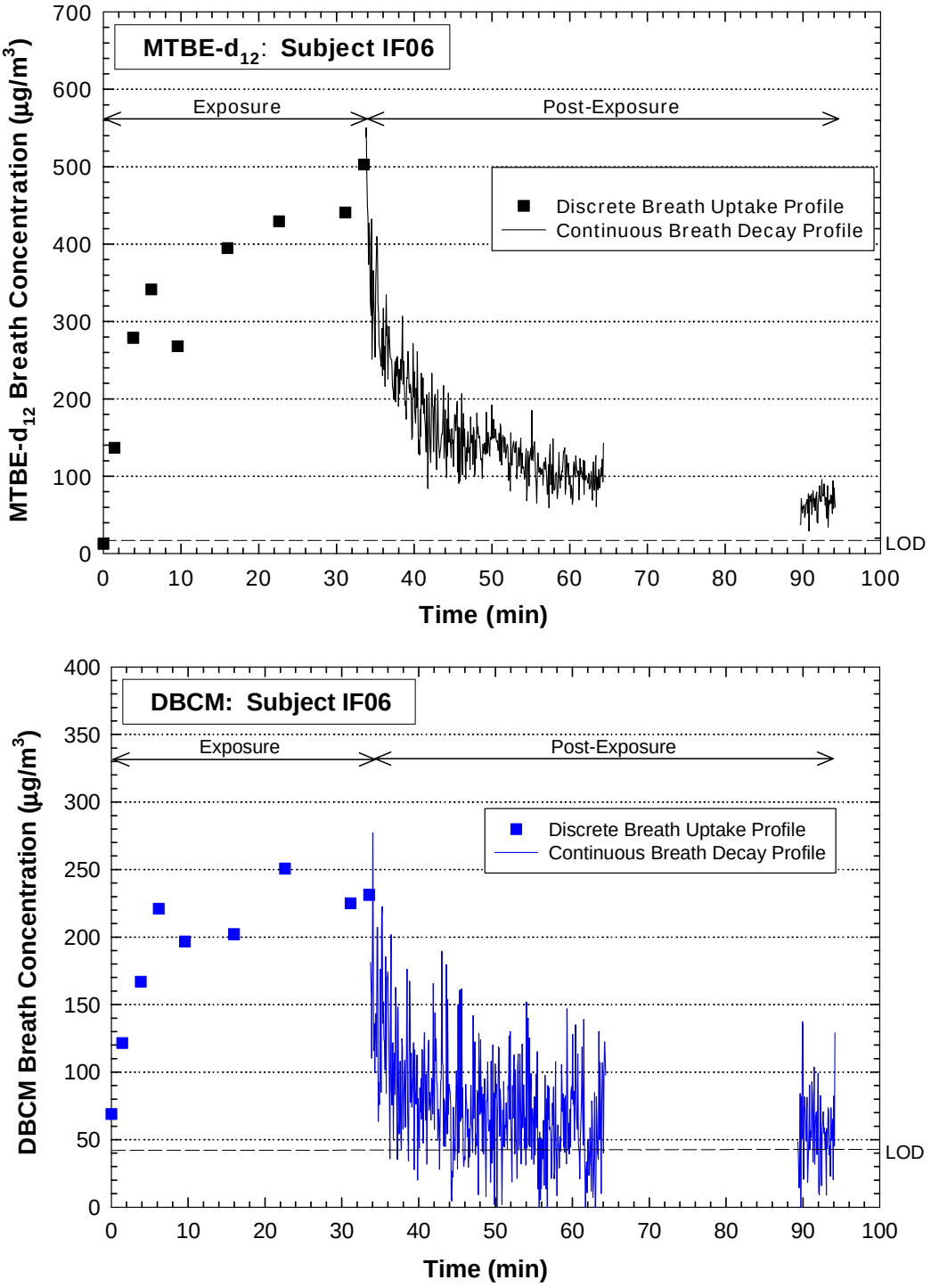

Figure 5-13. Discrete uptake and continuous decay profiles of MTBE-d_{12} (upper plot) and DBCM (lower plot) in breath for female Subject IF06 exposed to 2,217 µg/m^3 (542 ppbv) of MTBE-d_{12} and 728 µg/m^3 (85.6 ppbv) of DBCM in air for 30.7 minutes (effective exposure period). LOD designates limit of detection for target compound.

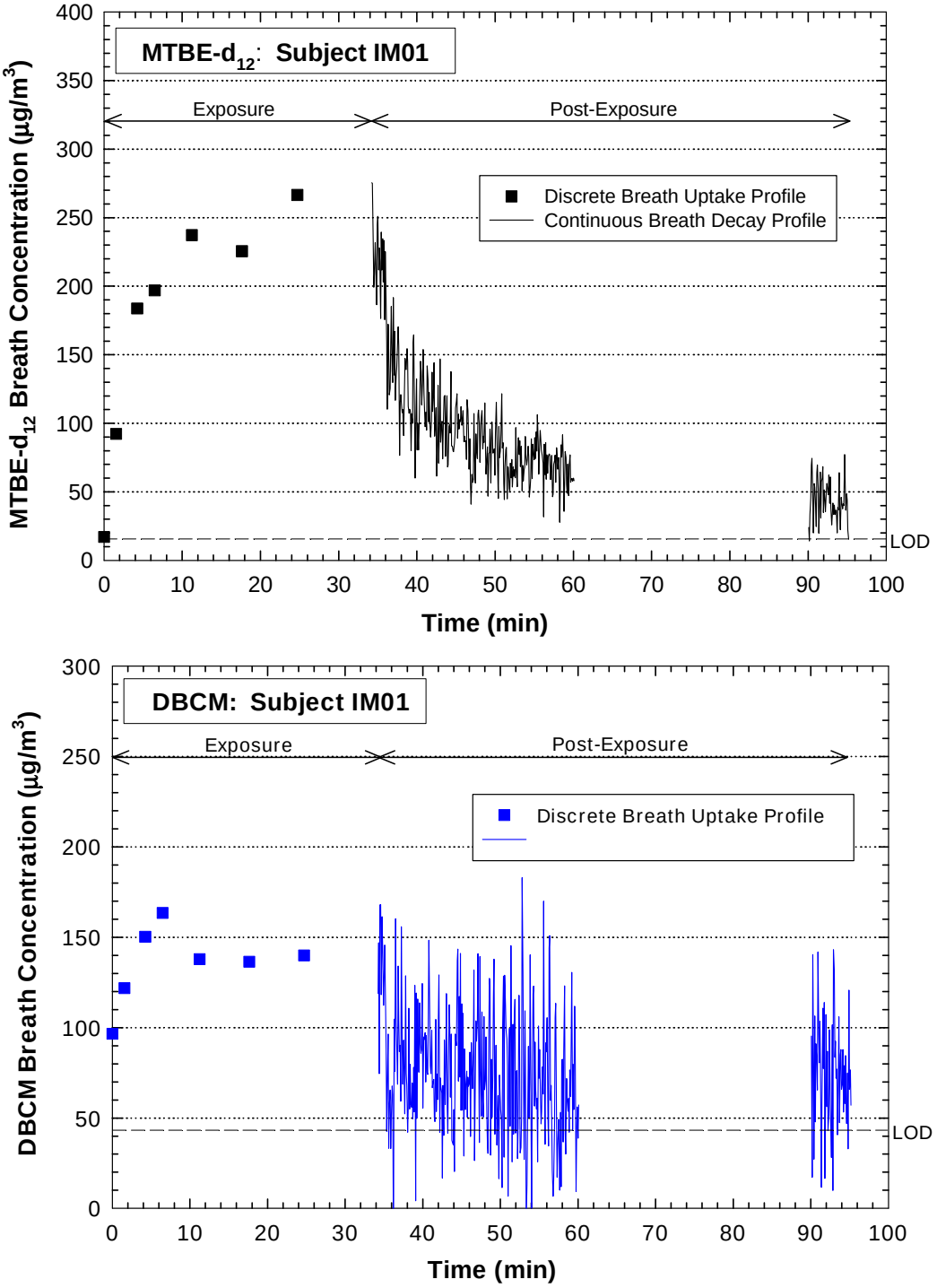

Figure 5-14. Discrete uptake and continuous decay profiles of MTBE-d_{12} (upper plot) and DBCM (lower plot) in breath for male Subject IM01 exposed to 2,217 µg/m^3 (542 ppbv) of MTBE-d_{12} and 728 µg/m^3 (85.6 ppbv) of DBCM in air for 30.5 minutes (effective exposure period). LOD designates limit of detection for target compound.

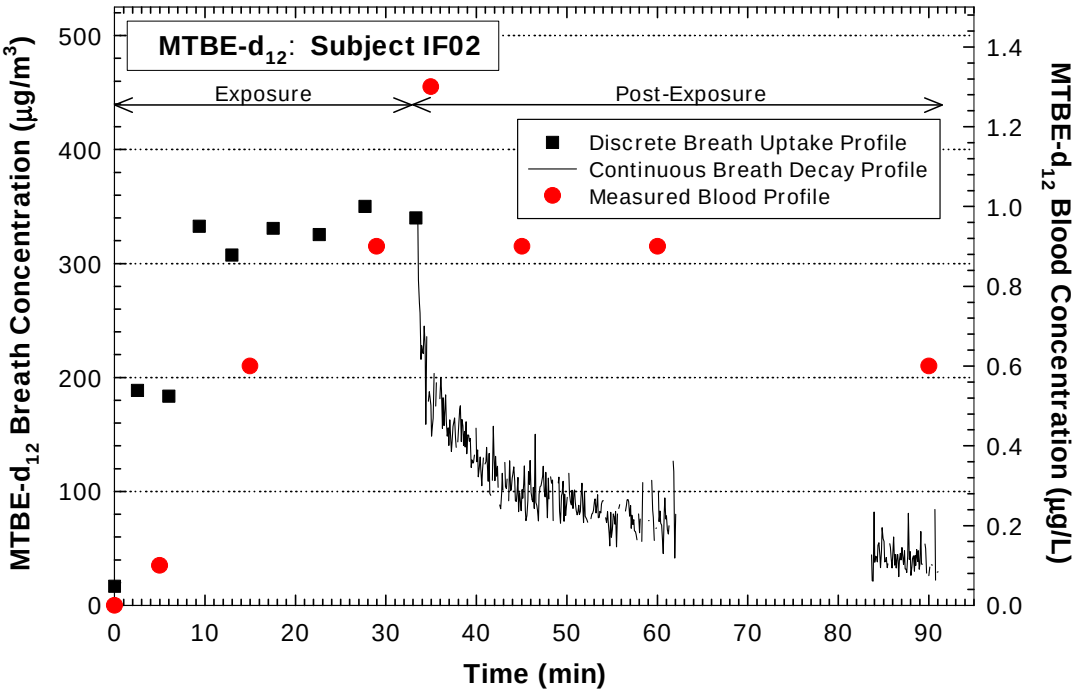

Figure 5-15. Uptake and decay of MTBE-d_{12} in breath and blood for female Subject IF02 exposed to 2,217 µg/m^3 (542 ppbv) of MTBE-d_{12} and 728 µg/m^3 (85.6 ppbv) of DBCM in air for 29.3 minutes.

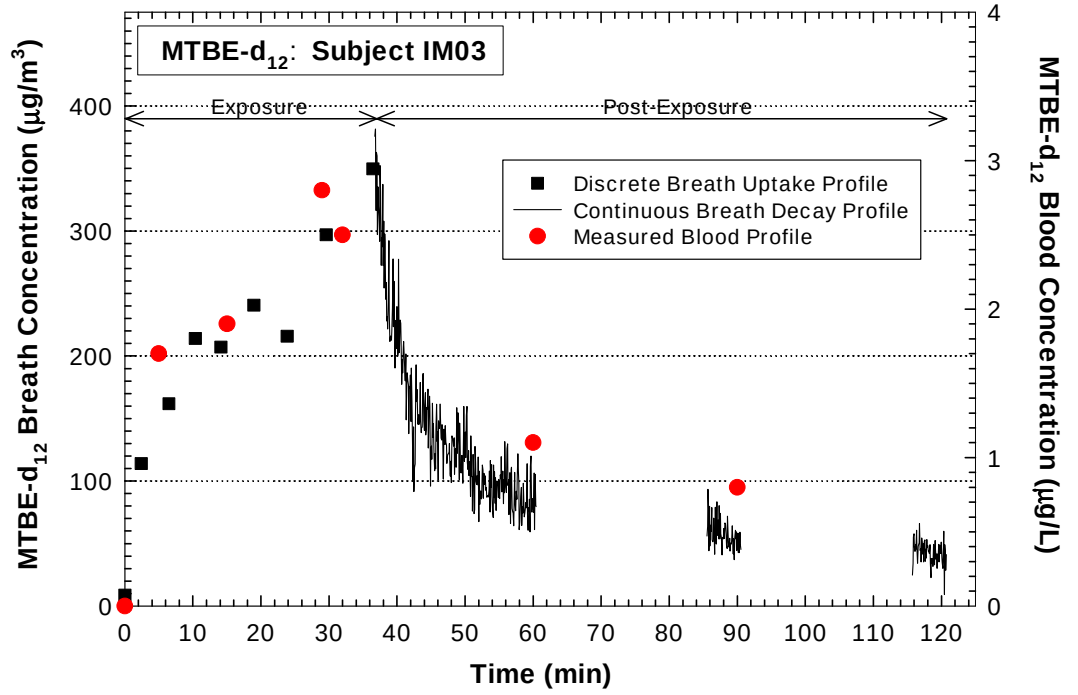

Figure 5-16. Uptake and decay of MTBE-d_{12} in breath and blood for male Subject IM03 exposed to 2,217 µg/m^3 (542 ppbv) of MTBE-d_{12} and 728 µg/m^3 (85.6 ppbv) of DBCM in air for 30.6 minutes.

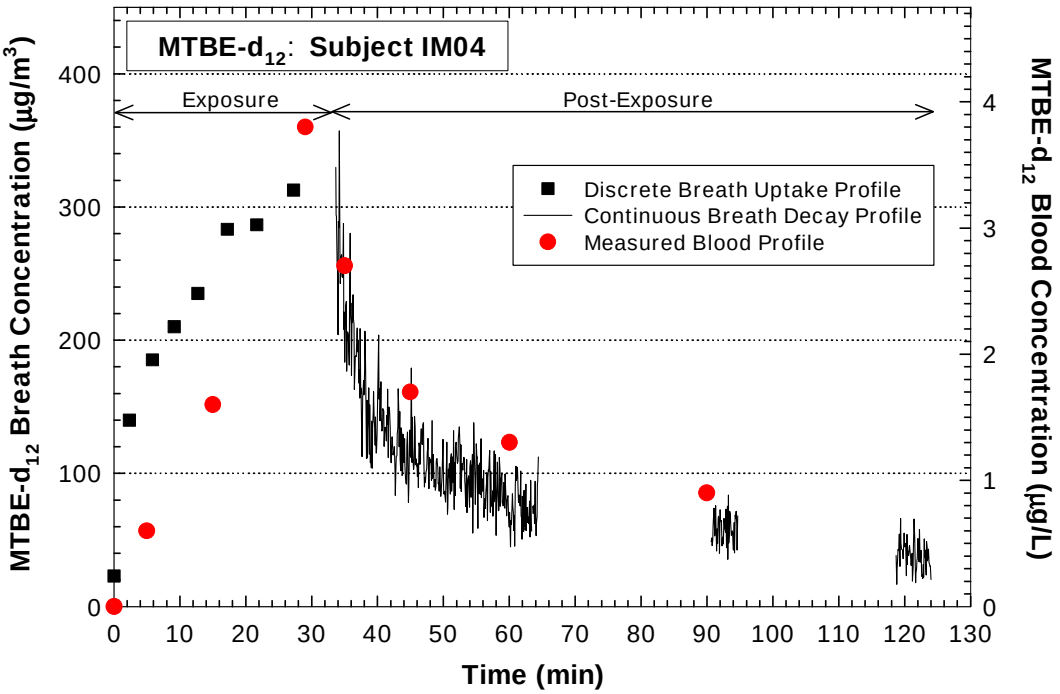

Figure 5-17. Uptake and decay of MTBE-d_{12} in breath and blood for male Subject IM04 exposed to 2,217 µg/m^3 (542 ppbv) of MTBE-d_{12} and 728 µg/m^3 (85.6 ppbv) of DBCM in air for 30.3 minutes.

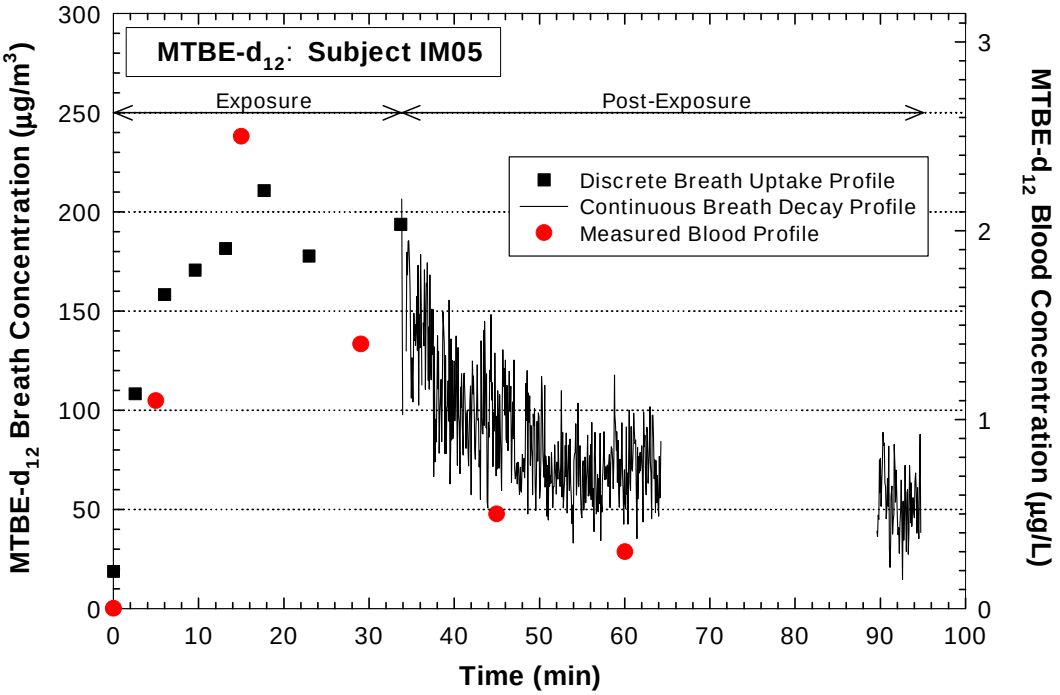

Figure 5-18. Uptake and decay of MTBE-d_{12} in breath and blood for male Subject IM05 exposed to 2,217 µg/m^3 (542 ppbv) of MTBE-d_{12} and 728 µg/m^3 (85.6 ppbv) of DBCM in air for 30.6 minutes.

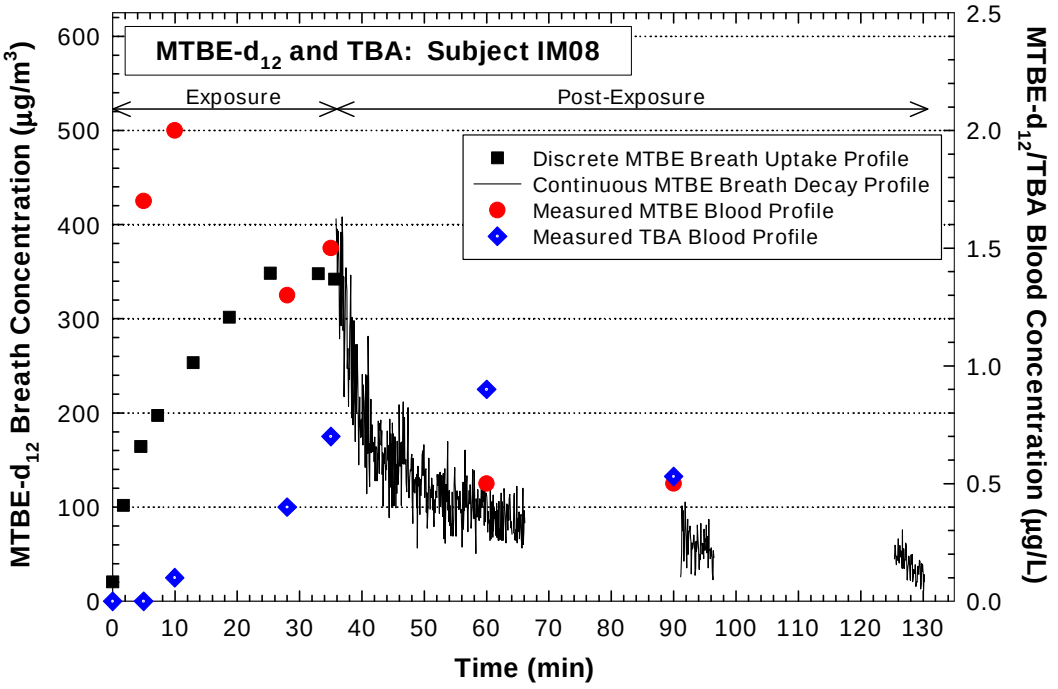

Figure 5-19. Uptake and decay of MTBE-d_{12} in breath and blood, and of TBA in blood, for male Subject IM08 exposed to 2,217 µg/m^3 (542 ppbv) of MTBE-d_{12} and 728 µg/m^3 (85.6 ppbv) of DBCM in air for 30.6 min.

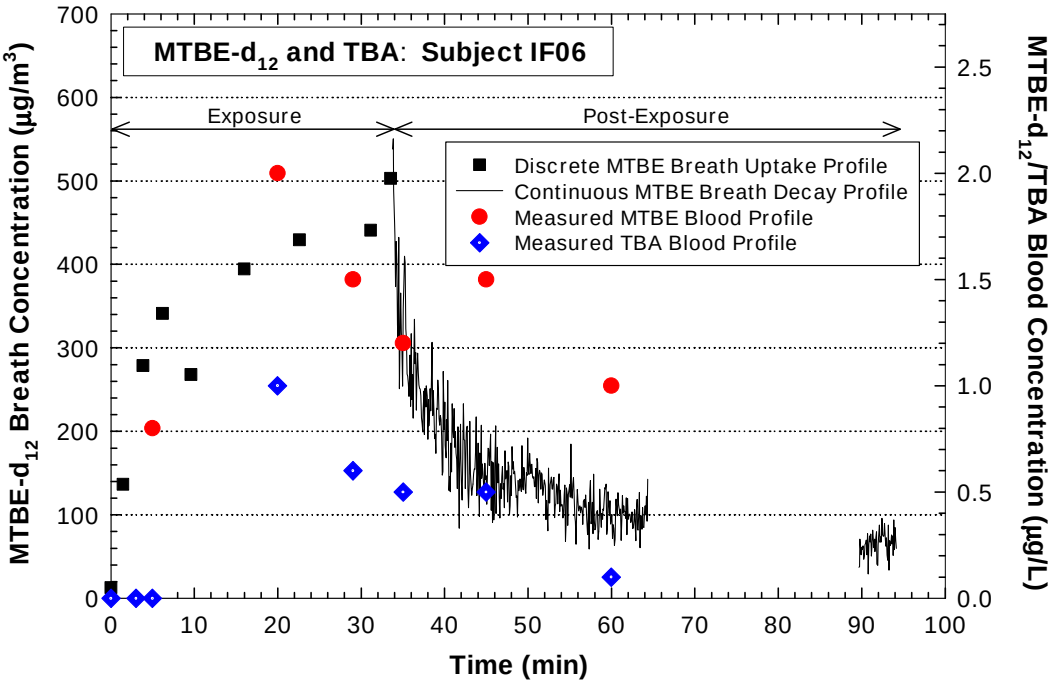

Figure 5-20. Uptake and decay of MTBE-d_{12} in breath and blood, and of TBA in blood, for female Subject IF06 exposed to 2,217 µg/m^3 (542 ppbv) of MTBE-d_{12} and 728 µg/m^3 (85.6 ppbv) of DBCM in air for 30.7 min.

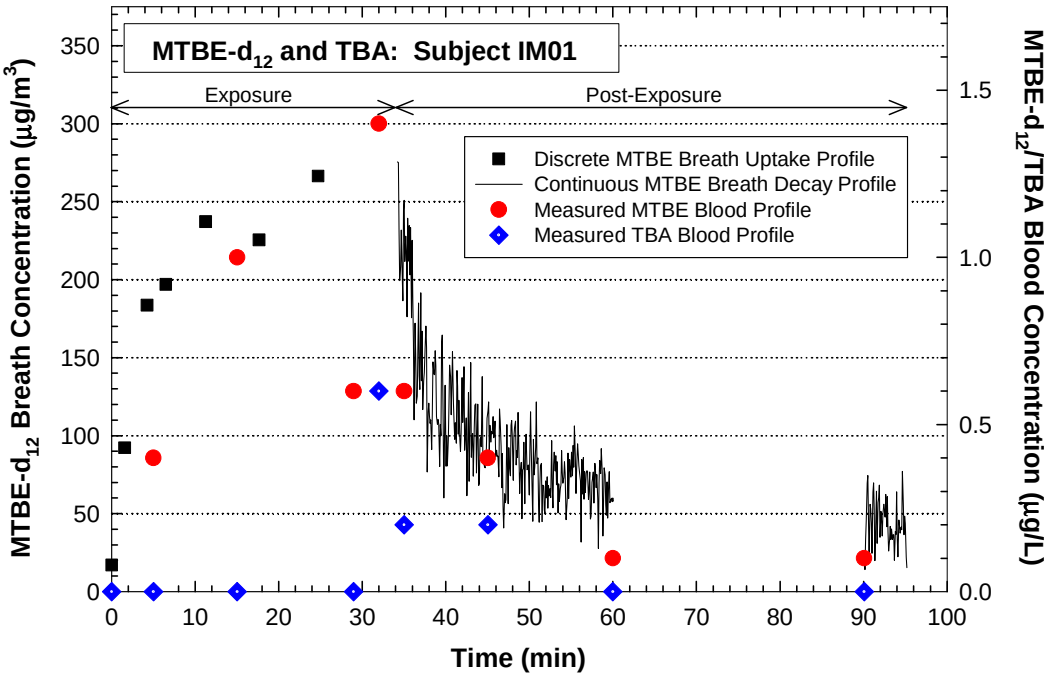

Figure 5-21. Uptake and decay of MTBE-d_{12} in breath and blood, and of TBA in blood, for male Subject IM01 exposed to 2,217 µg/m^3 (542 ppbv) of MTBE-d_{12} and 728 µg/m^3 (85.6 ppbv) of DBCM in air for 30.5 min.

Total Absorbed Dose

The MTBE-d_{12} total absorbed dose to each subject was calculated from Equations 4-1 to 4-10. Results are presented for all seven subjects in Table 5-2. The mean MTBE-d_{12} total absorbed dose was 148.5 ± 34.4 μg [mean ± standard deviation (SD)] for the ~30-min exposure.

We were unable to derive total absorbed dose values for DBCM from the exhaled breath profiles obtained for this target analyte (see Figures 5-8 to 5-14). In most cases, the measured signals were barely above the limit of detection, giving rise to considerable uncertainty in the curve fitting phase. In addition, the decay portions of the profiles appeared to decrease unusually slowly, suggesting that the measured signal may, in fact, have been affected by an unknown contaminant. This slow decrease during elimination had the effect of greatly increasing the estimated areas under the decay curves such that the resultant values were effectively meaningless.

Fraction of Compound Exhaled Unchanged at Equilibrium

The fraction of MTBE-d_{12} eliminated through respiration at equilibrium was estimated as the ratio of the "unmetabolized mass" to the total ("applied") dose (Equation (4-12)). The "unmetabolized mass" was calculated from the product of the total area under the uptake and decay curves with the alveolar ventilation rate (Equation (4-11)). Finally, the total dose was determined from the product of the total exposure and the subject's average alveolar ventilation rate (Equation (4-1)). Results for the value of *f* obtained in this way for MTBE-d_{12} are summarized in Table 5-2; the mean value of *f* was 0.29 ± 0.04. As explained above, we were unable to estimate a value of *f* for DBCM from the exhaled breath data in Figures 5-8 to 5-14.

Empirical Modeling of Uptake and Decay Breath and Blood Concentrations

The linear compartment model developed by Wallace et al.[29] was used to model the MTBE-d_{12} uptake and decay concentrations in the breath and blood of the participants. Equations (4-16) and (4-18) were fitted to the observed data. Curve fitting to estimate the coefficients in the equations was accomplished using SigmaPlot. The results for MTBE-d_{12} are shown in Figures 5-22 to 5-35, and values obtained for the calculated uptake and elimination parameters are presented in Table 5-3. For the breath and blood uptake phase, a one-compartment model was assumed; for the elimination phase, we used a two-compartment model for the breath data and a one-compartment model for the blood data.

The results obtained by applying the linear compartment model to the breath and blood uptake and decay data for DBCM are summarized in Table 5-4. As already noted, in most cases for DBCM, the measured signals were only slightly above the detection limit, which resulted in considerable uncertainty in the curve fitting. This uncertainty is clearly reflected in the data presented in Table 5-4.

Table 5-2. Total absorbed dose of MTBE-d_{12} as a result of inhalation exposure.

Parameter	Subj. IF02	Subj. IM03	Subj. IM04	Subj. IM05	Subj. IM08	Subj. IF06	Subj. IM01	Mean	Std Dev
Total Exposure									
C_{air} (µg/m^3)	2,217	2,217	2,217	2,217	2,217	2,217	2,217	**2,217**	**0**
Total Elapsed Exposure Time, *T* (min)	33.3	36.7	33.5	33.9	35.8	33.8	34.2	**34.5**	**1.3**
Total Interrupted MTBE Uptake Period (min)	3.97	6.08	3.25	3.26	5.20	3.14	3.68	**4.08**	**1.13**
Effective Exposure Time, *T′* (min)	29.3	30.6	30.3	30.6	30.6	30.7	30.5	**30.4**	**0.5**
Total Exposure, E_{total} (µg.min/m^3)	64,958	67,840	67,175	67,840	67,840	68,062	67,619	**67,333**	**1,084**
Uptake Ventilation Rate (L/min)	3.49	5.97	4.90	4.74	4.56	5.56	3.08	**4.61**	**1.04**
Uptake Alveolar Ventilation Rate, *AVR* (L/min)[a]	2.34	4.00	3.28	3.18	3.06	3.73	2.06	**3.09**	**0.69**
Total ("Applied") Dose (µg)	152.0	271.4	220.3	215.7	207.6	253.9	139.3	**208.6**	**48.6**
Total Amount Exhaled During Uptake Period									
Uptake Period, *T* (min)	33.3	36.7	33.5	33.9	35.8	33.8	34.2	**34.5**	**1.3**
Uptake *AVR* (L/min)[a]	2.34	4.00	3.28	3.18	3.06	3.73	2.06	**3.09**	**0.69**
Area Under Uptake Curve (µg.min/m^3)	9789	8216	8132	5816	9679	12615	7041	**8755**	**2201**
Total Amount Exhaled During Uptake (µg)	22.9	32.9	26.7	18.5	29.6	47.1	14.5	**27.4**	**10.7**
Total Amount Exhaled During Decay Period									
Monitored Decay Period (min)	57.4	83.5	90.4	60.8	94.2	60.1	60.6	**—**	**—**
Decay Ventilation Rate (L/min)	3.69	6.13	5.70	4.45	7.02	4.49	4.27	**5.11**	**1.20**
Decay Alveolar Ventilation Rate, *AVR* (L/min)[a]	2.47	4.11	3.82	2.98	4.70	3.01	2.86	**3.42**	**0.80**
a_1 (µg/m^3)[b]	111.8	225.5	154.0	73.0	229.6	247.9	141.9	**169.1**	**66.5**
τ_{1decay} (min)[b]	2.39	3.91	3.81	7.82	3.41	2.68	2.73	**3.82**	**1.86**
a_2 (µg/m^3)[b]	148.7	123.4	122.5	77.0	143.4	182.7	102.8	**128.6**	**34.0**
τ_{2decay} (min)[b]	45.7	70.9	72.5	161.3	64.1	50.8	63.3	**75.5**	**39.1**
Area Under Decay Curve (µg.min/m^3)[c]	7063	9631	9468	12991	9975	9946	6895	**9424**	**2054**
Total Amount Exhaled During Decay (µg)	17.5	39.6	36.2	38.7	46.9	29.9	19.7	**32.6**	**10.8**

Table 5-2. Total absorbed dose of MTBE-d_{12} as a result of inhalation exposure (continued).

Parameter	Subj. IF02	Subj. IM03	Subj. IM04	Subj. IM05	Subj. IM08	Subj. IF06	Subj. IM01	Mean	Std Dev
Total Absorbed Dose									
Total Exhaled During Uptake + Decay (µg)	40.4	72.4	62.8	57.2	76.5	77.0	34.2	**60.1**	**17.2**
Total Absorbed ("Internal") Dose = Total Dose – Unmetabolized Mass (µg)	111.6	199.0	157.5	158.5	131.1	176.9	105.1	**148.5**	**34.4**
Fraction *f* Exhaled at Equilibrium	0.27	0.27	0.29	0.27	0.37	0.30	0.25	**0.29**	**0.04**
Fraction Absorbed (= 1 – *f*)	0.74	0.74	0.74	0.72	0.71	0.67	0.79	**0.73**	**0.04**
Fraction Eliminated Post-Exposure (0-90 min)	0.311	0.391	0.461	0.300	0.743[d]	0.387	0.346	**0.366**	**0.060**
Average Uptake/Decay AVR (L/min)	2.41	4.06	3.55	3.08	3.88	3.37	2.46	**3.26**	**0.65**
From Model: Total Absorbed Dose $AVR.C_{air}.T'.(1-f)$	115.0	201.9	170.5	153.5	166.2	159.8	125.5	**156.1**	**29.0**

[a] Alveolar ventilation rate assumed to be 67% of ventilation rate.
[b] Determined from nonlinear curve fit to exponential decay model (see Table 5-3).
[c] Estimated from Equation (4-9):[49] $AUC_{decay} = \int C_{alv}(t)dt = \sum a_i \tau_i$.
[d] Excluded from calculation of the mean.

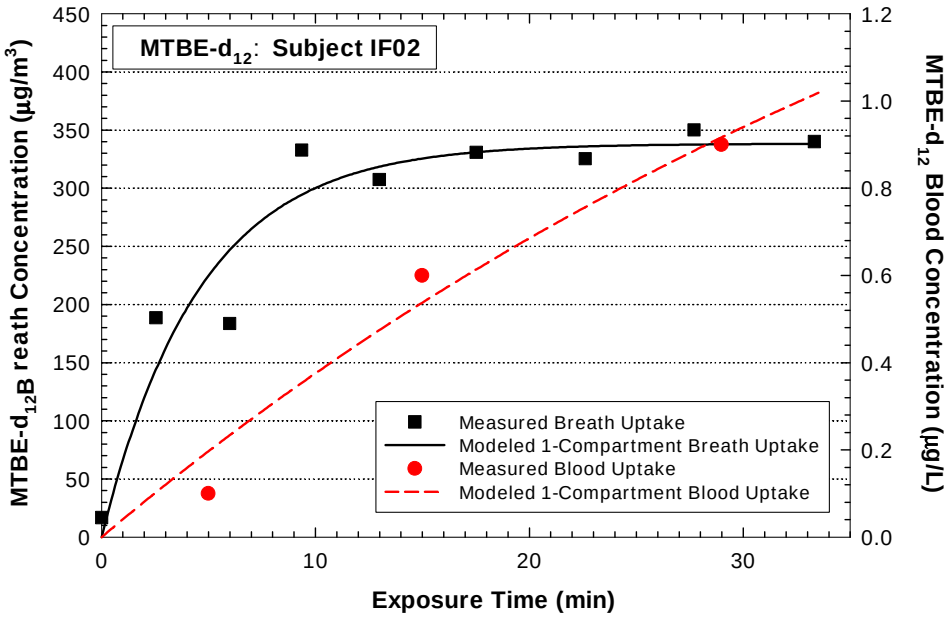

Figure 5-22. Measured and modeled uptake of MTBE-d_{12} in exhaled breath and venous blood for female Subject IF02 exposed to 2,217 µg/m^3 (542 ppbv) of MTBE-d_{12} in air for 29.3 minutes.

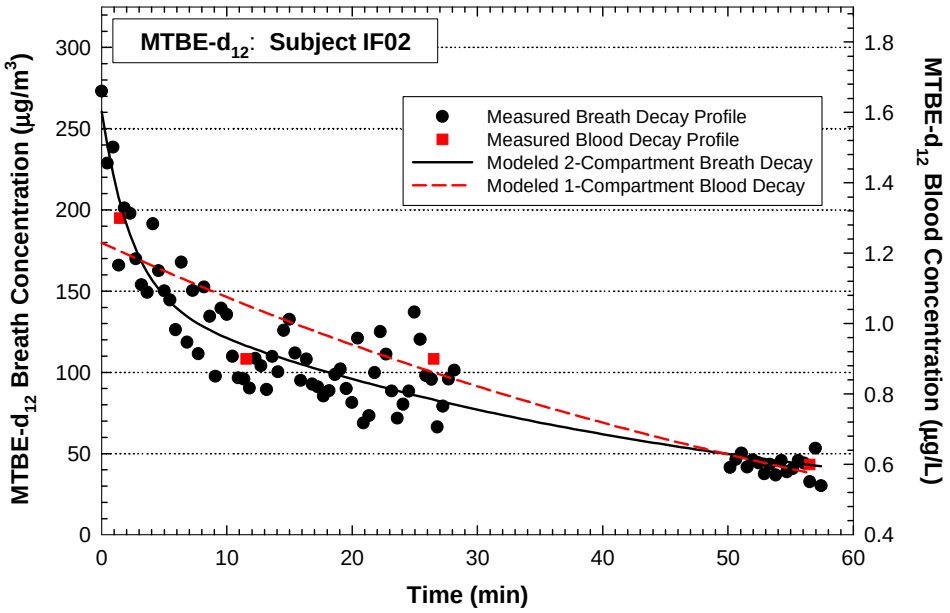

Figure 5-23. Measured and modeled elimination of MTBE-d_{12} from exhaled breath and venous blood for female Subject IF02 after exposure to 2,217 µg/m^3 (542 ppbv) of MTBE-d_{12} in air for 29.3 minutes. Breath data smoothed using 5-point moving average.

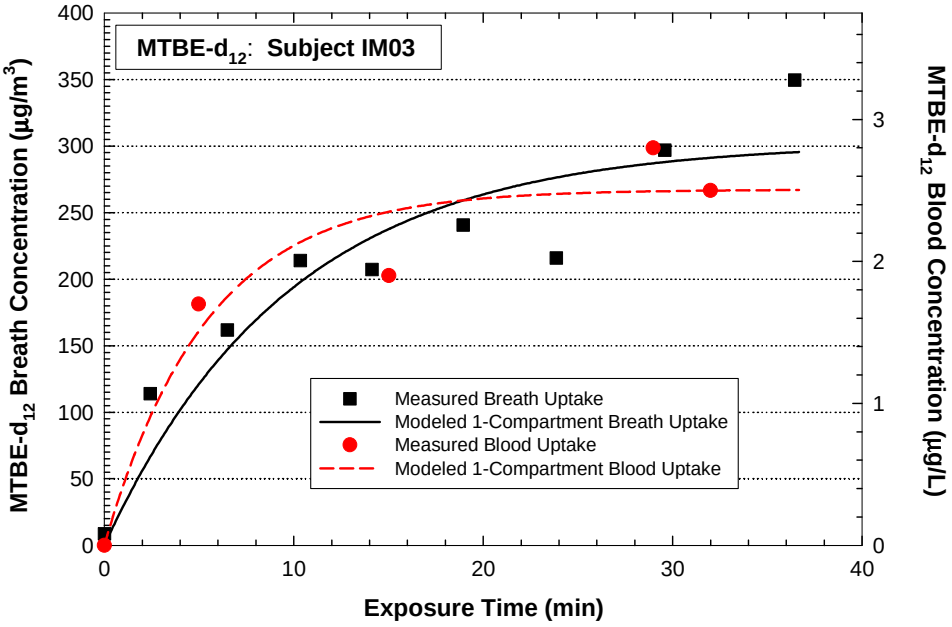

Figure 5-24. Measured and modeled uptake of MTBE-d_{12} in exhaled breath and venous blood for male Subject IM03 exposed to 2,217 µg/m^3 (542 ppbv) of MTBE-d_{12} in air for 30.6 minutes.

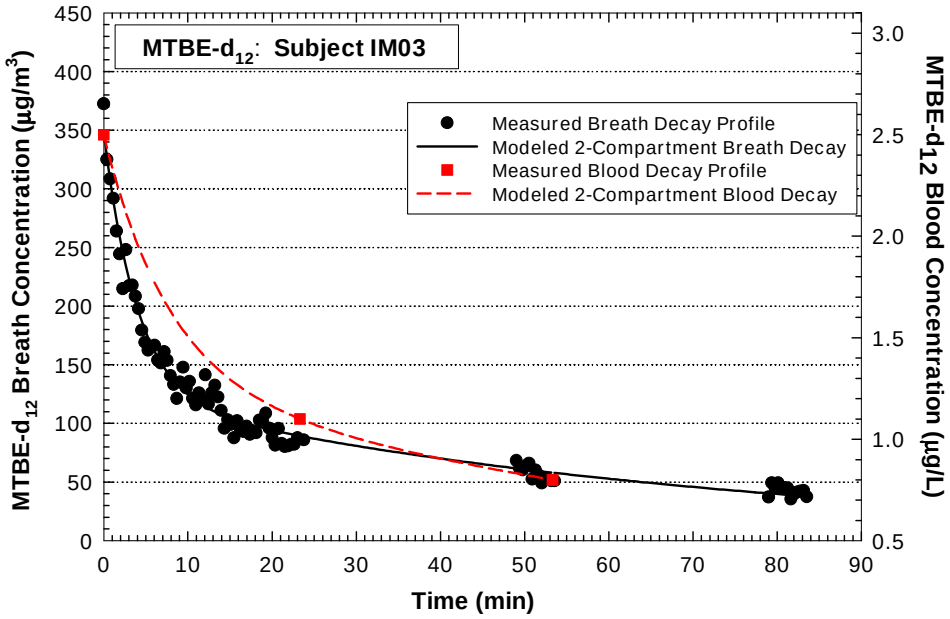

Figure 5-25. Measured and modeled elimination of MTBE-d_{12} from exhaled breath and venous blood for male Subject IM03 after exposure to 2,217 µg/m^3 (542 ppbv) of MTBE-d_{12} in air for 30.6 minutes. Breath data smoothed using 5-point moving average.

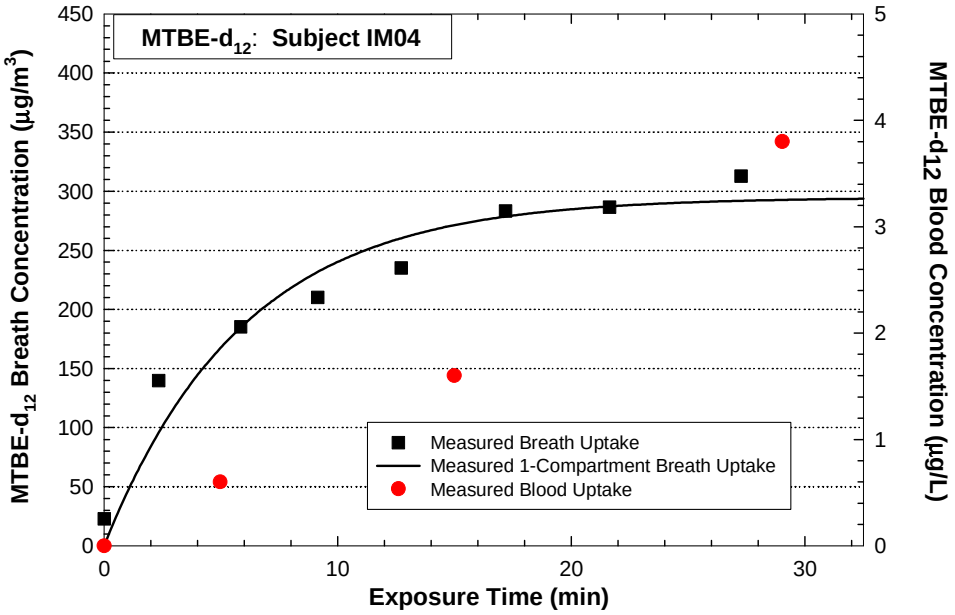

Figure 5-26. Measured and modeled uptake of MTBE-d_{12} in exhaled breath and venous blood for male Subject IM04 exposed to 2,217 µg/m^3 (542 ppbv) of MTBE-d_{12} in air for 30.3 minutes.

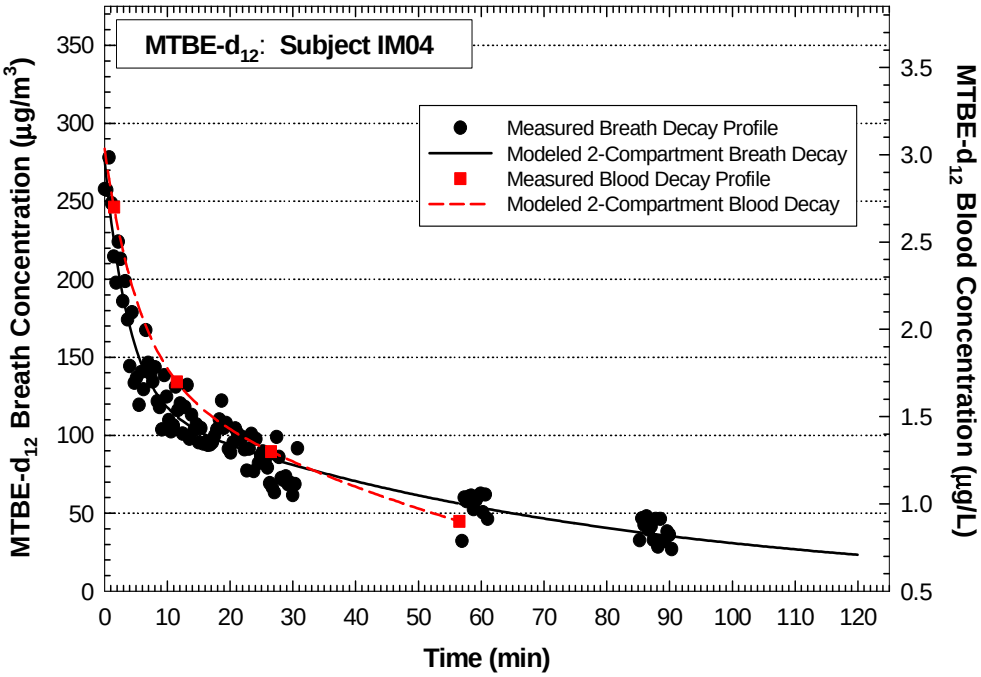

Figure 5-27. Measured and modeled elimination of MTBE-d_{12} from exhaled breath and venous blood for male Subject IM04 after exposure to 2,217 µg/m^3 (542 ppbv) of MTBE-d_{12} in air for 30.3 minutes. Breath data smoothed using 5-point moving average.

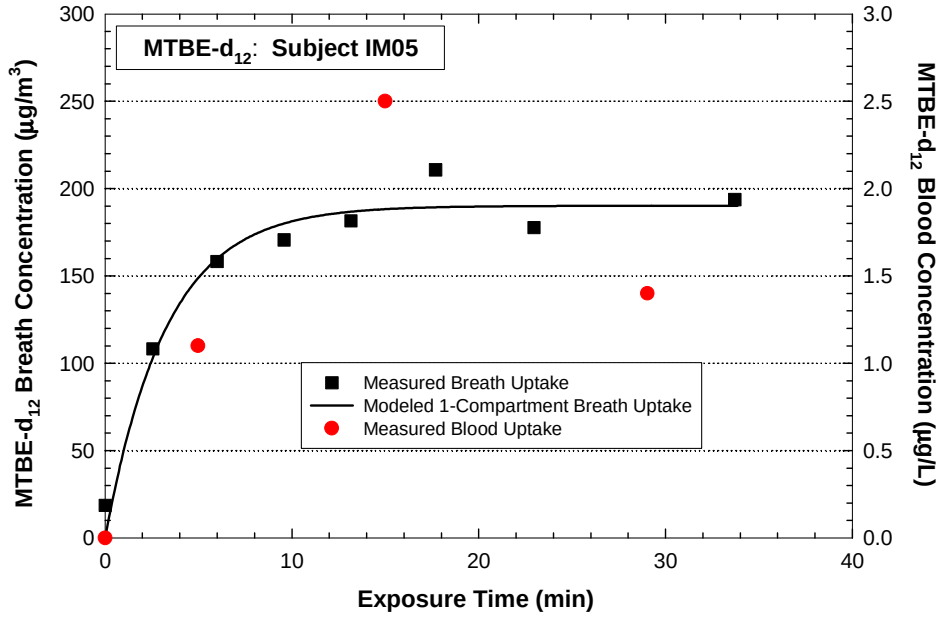

Figure 5-28. Measured and modeled uptake of MTBE-d_{12} in exhaled breath and venous blood for male Subject IM05 exposed to 2,217 µg/m^3 (542 ppbv) of MTBE-d_{12} in air for 30.6 minutes.

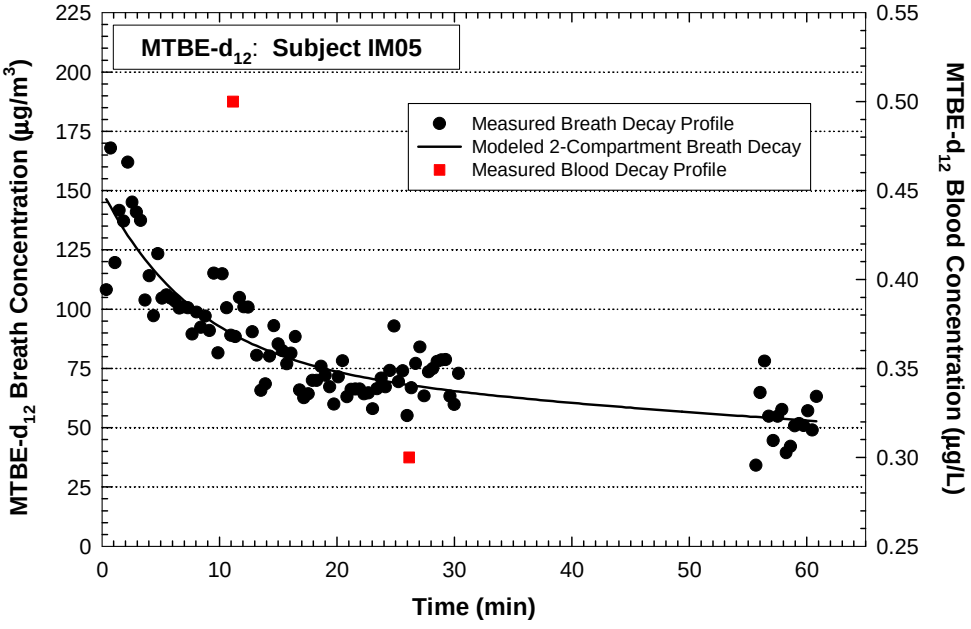

Figure 5-29. Measured and modeled elimination of MTBE-d_{12} from exhaled breath and venous blood for male Subject IM05 after exposure to 2,217 µg/m^3 (542 ppbv) of MTBE-d_{12} in air for 30.6 minutes. Breath data smoothed using 5-point moving average.

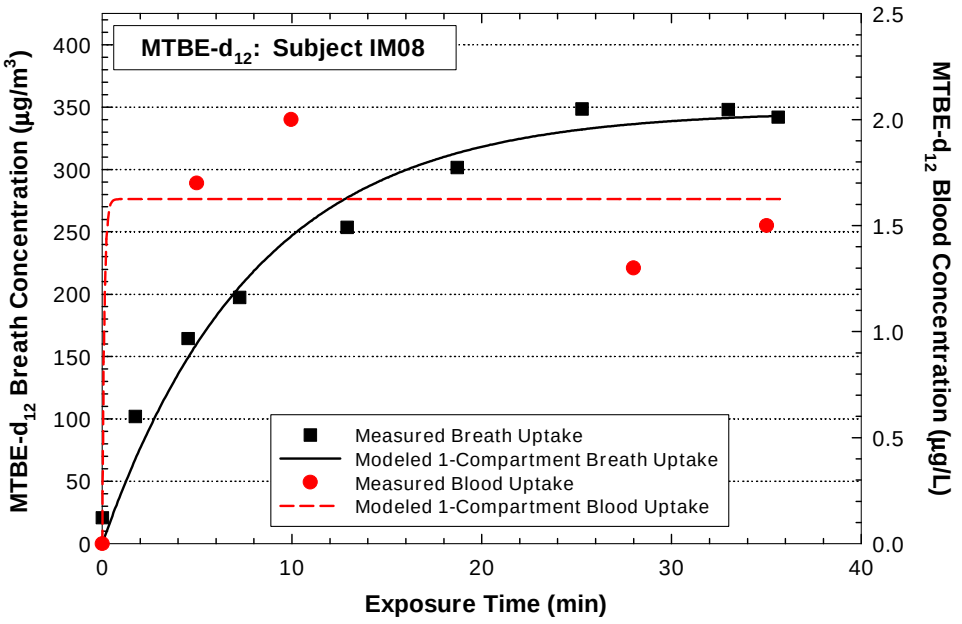

Figure 5-30. Measured and modeled uptake of MTBE-d_{12} in exhaled breath and venous blood for male Subject IM08 exposed to 2,217 µg/m^3 (542 ppbv) of MTBE-d_{12} in air for 30.6 minutes.

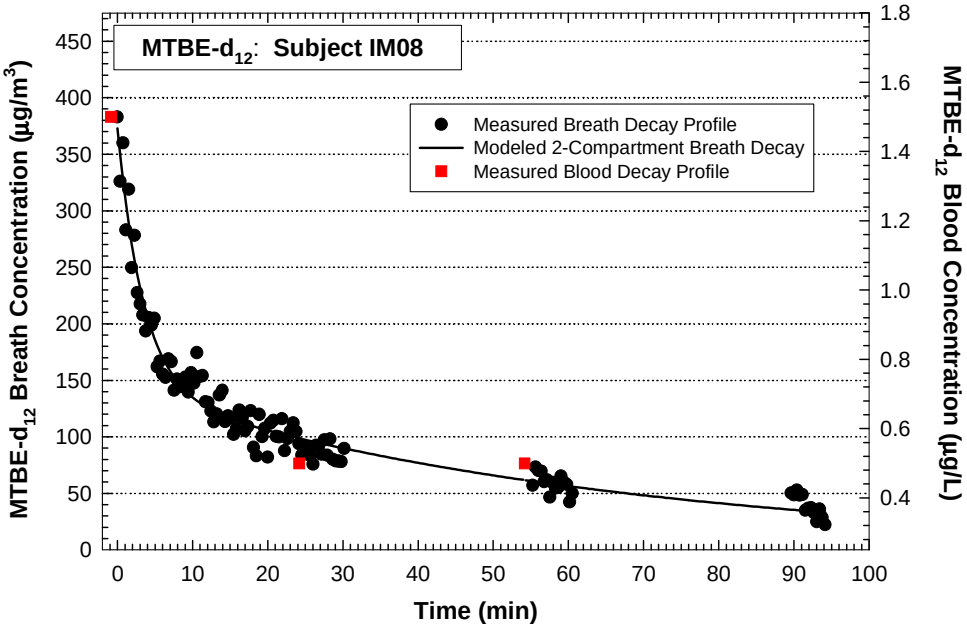

Figure 5-31. Measured and modeled elimination of MTBE-d_{12} from exhaled breath and venous blood for male Subject IM08 after exposure to 2,217 µg/m^3 (542 ppbv) of MTBE-d_{12} in air for 30.6 minutes. Breath data smoothed using 5-point moving average.

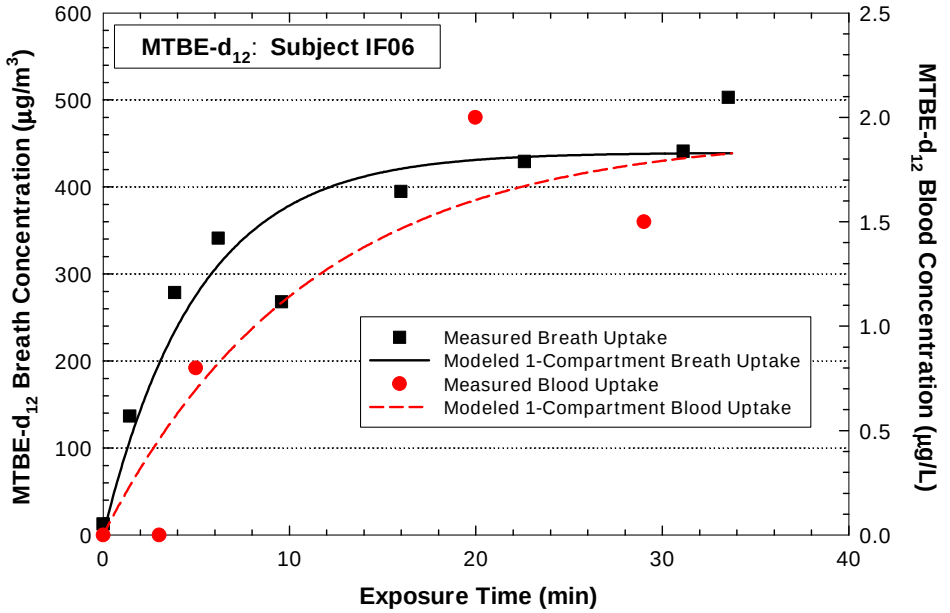

Figure 5-32. Measured and modeled uptake of MTBE-d_{12} in exhaled breath and venous blood for female Subject IF06 exposed to 2,217 µg/m^3 (542 ppbv) of MTBE-d_{12} in air for 30.7 minutes.

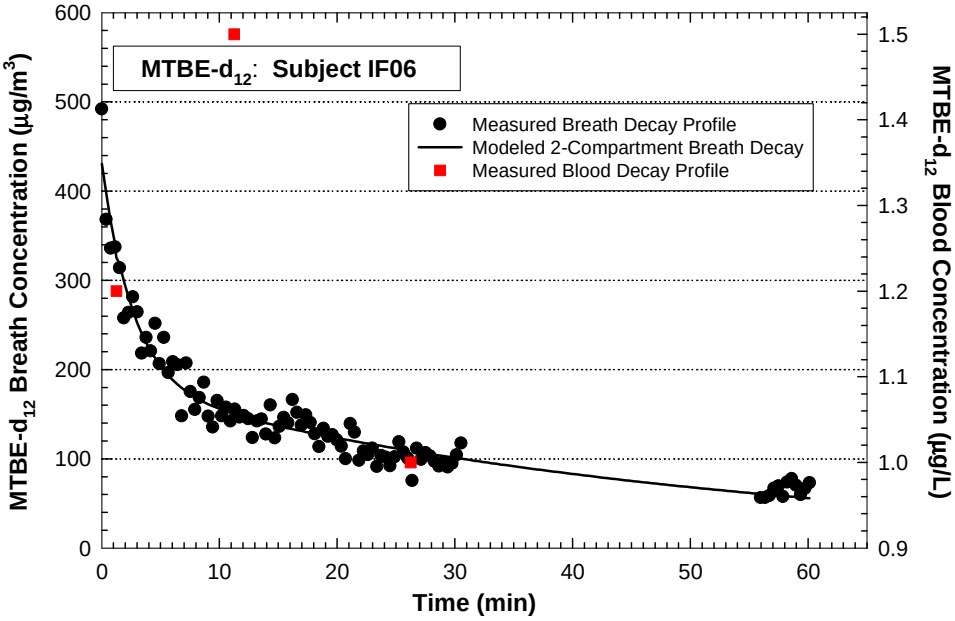

Figure 5-33. Measured and modeled elimination of MTBE-d_{12} from exhaled breath and venous blood for female Subject IF06 after exposure to 2,217 µg/m^3 (542 ppbv) of MTBE-d_{12} in air for 30.7 minutes. Breath data smoothed using 5-point moving average.

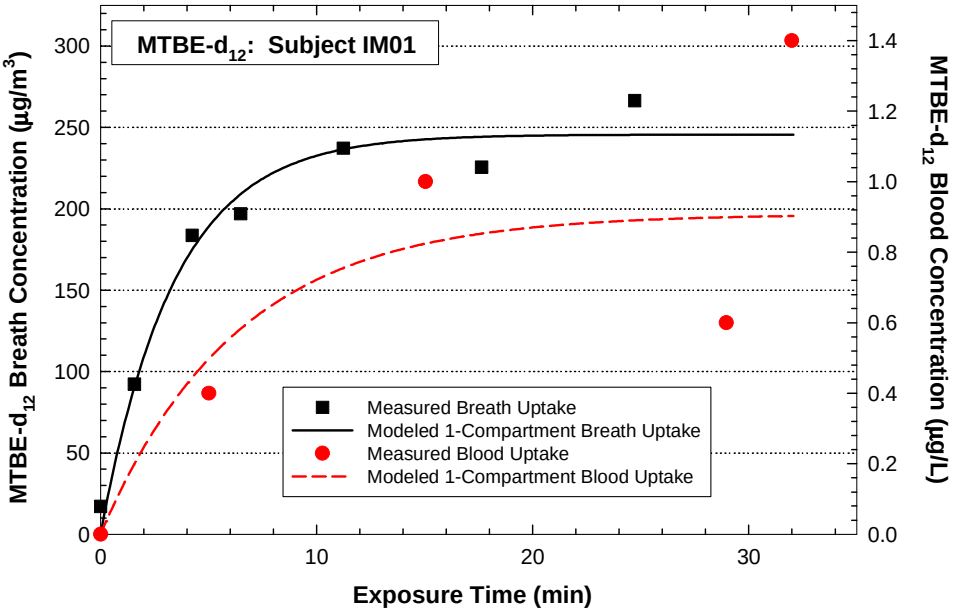

Figure 5-34. Measured and modeled uptake of MTBE-d_{12} in exhaled breath and venous blood for male Subject IM01 exposed to 2,217 µg/m^3 (542 ppbv) of MTBE-d_{12} in air for 30.5 minutes.

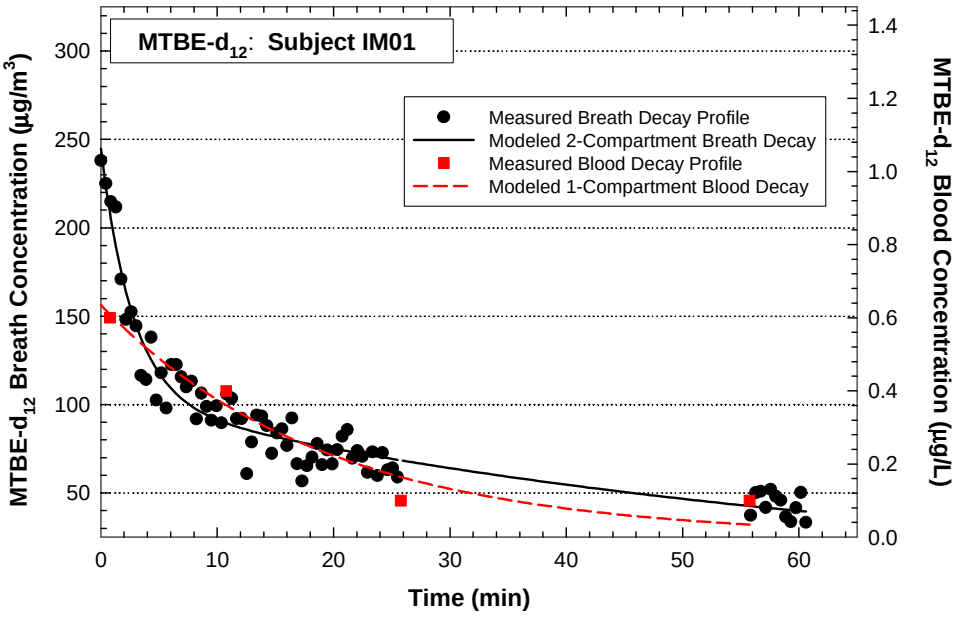

Figure 5-35. Measured and modeled elimination of MTBE-d_{12} from exhaled breath and venous blood for male Subject IM01 after exposure to 2,217 µg/m^3 (542 ppbv) of MTBE-d_{12} in air for 30.5 minutes. Breath data smoothed using 5-point moving average.

Table 5-3. Theoretical calculations of MTBE-d_{12} model parameters.

Matrix	Parameter	Subj. IF02	Subj. IM03	Subj. IM04	Subj. IM05	Subj. IM08	Subj. IF06	Subj. IM01	Mean	Std Dev
	C_{air} (µg/m^3)	2,217	2,217	2,217	2,217	2,217	2,217	2,217	**2,217**	
	Exposure Time (min)	29.3	30.6	30.3	30.6	30.6	30.7	30.5	**30.4**	**0.5**
	Uptake Models									
Breath										
	$fC_{air}a_1$ (= Max. Breath Concn.) (µg/m^3)	338[a]	303[a]	295[a]	190[a]	347[a]	440[a]	246[a]	**308**	**79**
	$\tau_{1uptake}$	4.58[b]	9.76[c]	5.94[b]	3.26[b]	8.05[a]	5.06[b]	3.41[b]	**5.7**	**2.4**
	Adjusted R^2 [d]	0.908	0.849	0.926	0.951	0.970	0.885	0.967		
Blood										
	$fC_{air}a_1$	2.13[e]	2.51[c]	nc[h]	1.92[e]	1.63[b]	1.92[e]	0.91[c]	**1.84**	**0.54**
	$\tau_{1uptake}$	(51.6[e])	5.41[e]	nc[h]	4.54[e]	0.11[e]	11.04[e]	6.32[e]	**5.5**	**3.9**
	Adjusted R^2 [d]	0.962	0.907	nc[k]	0.658	0.850	0.810	0.499		
	Elimination Models									
Breath										
	Max. Breath Conc. (µg/m^3)	260	349	277	150	373	431	245	**298**	**94**
	$_1$	112[a]	226[a]	154[a]	73.0[a]	230[a]	248[a]	142[a]	**169**	**67**
	$_2$	149[a]	123[a]	123[a]	77.0[a]	143[a]	183[a]	103[a]	**129**	**34**
	τ_{1decay}	2.39[a]	3.91[a]	3.81[a]	7.82[a]	3.41[a]	2.68[a]	2.73[a]	**3.8**	**1.9**
a	τ_{2decay}	45.7[a]	70.9[a]	72.5[a]	(161.3[c])	64.1[a]	50.8[a]	63.3[a]	**61**	**11**
a	Adjusted R^2 [d]	0.884	0.982	0.947	0.802	0.965	0.949	0.943		
Blood										
	$_1$	1.23[b]	1.23[a,f]	1.26[a,g]	—[i]	—[i]	—[i]	0.63[c]	**1.1**	**0.3**
	$_2$		1.27[a,f]	1.77[a,g]	—[i]	—[i]	—[i]	—	—	—
	τ_{1decay}	(74.6[e])	7.78[a,f]	5.50[a,g]	—[i]	—[i]	—[i]	19.1[e]	**10.8**	**7.3**
a	τ_{2decay}		114[a,f]	83.3[a,g]	—[i]	—[i]	—[i]	—	—	—
	Adjusted R^2 [d]	0.791	>0.999	>0.999	—[i]	—[i]	—[i]	0.916		

[a] Highly significant (p <0.0005) value.
[b] Significant (p <0.005) value.
[c] Significant (p <0.05) value.
[d] Adjusted R^2 is the adjusted coefficient of determination, which takes into account the number of independent variables.
[e] Not a significant (p >0.05) value.
[f] Based on only three measured values.
[g] Based on only four measured values.
[h] nc = no convergence.
[i] Only two measured blood values available.

Table 5-4. Theoretical calculations of DBCM model parameters.

Matrix	Parameter	Subj. IF02	Subj. IM03	Subj. IM04	Subj. IM05	Subj. IM08	Subj. IF06	Subj. IM01	Mean	Std Dev
	C_{air} (µg/m^3)	728	728	728	728	728	728	728	**728**	
	Exposure Time (min)	29.3	30.6	30.3	30.6	30.6	30.7	30.5	**30.4**	**0.5**
	Uptake Models									
Breath										
	$fC_{air}a_1$	—	127[a]	—	171[a]	187[a]	222[a]	146[a]		
	$\tau_{1uptake}$	—	1.36[c]	—	1.59[c]	2.27[c]	2.16[b]	0.84[c]		
	Adjusted R^{2d}	—	0.000	—	—	—	0.700	—		
	Max. Breath Conc. (µg/m^3)	—	127	—	171	187	222	146		
	Elimination Models									
Breath										
	$_1$	92.0[b]	46.2[a]	80.0[a]	43.2[b]	145[a]	90.6[a]	66.6[a]		
	$_2$	80.8[a]	69.4[a]	102[a]	81.7[a]	72.0[a]	72.1[a]	76.1[a]		
	τ_{1decay}	1.55[c]	3.42[b]	6.54[a]	9.24[c]	1.86[a]	3.48[a]	0.62[c]		
a	τ_{2decay}	—	303[b]	833[c]	—	333[b]	222[c]	714[c]		
a	Adjusted R^{2} [d]	0.086	0.341	0.583	0.199	0.671	0.614	0.200		
	Max. Breath Conc. (µg/m^3)	173	116	182	125	217	163	143		

[a] Highly significant ($p < 0.0005$) value.
[b] Significant ($p < 0.05$) value.
[c] Not a significant ($p > 0.05$) value.
[d] Adjusted R^2 is the adjusted coefficient of determination, which takes into account the number of independent variables.

Relationship Between Breath and Blood Concentrations

As indicated earlier, in three cases (for Subjects IM03, IM05, and IM01, Figures 5-16, 5-18, and 5-21) we found that the blood levels for MTBE-d_{12} closely track the breath concentrations, indicating strong correlation between the blood and breath measurements. In these cases, the correlation coefficient R^2 ranged from 0.76 to 0.90. Figure 5-36 presents the plot of the breath MTBE-d_{12} versus the blood MTBE-d_{12} concentrations for Subject IM03 along with the linear regression-fitted curve, whose slope provides an estimate of the average venous blood-to-breath ratio. Two of the remaining four data sets yielded coefficients of 0.56 and 0.57, but for the last two, the values were only 0.29 and 0.07. Table 5-5 summarizes the correlation coefficients and blood-to-breath ratios established from the plots.

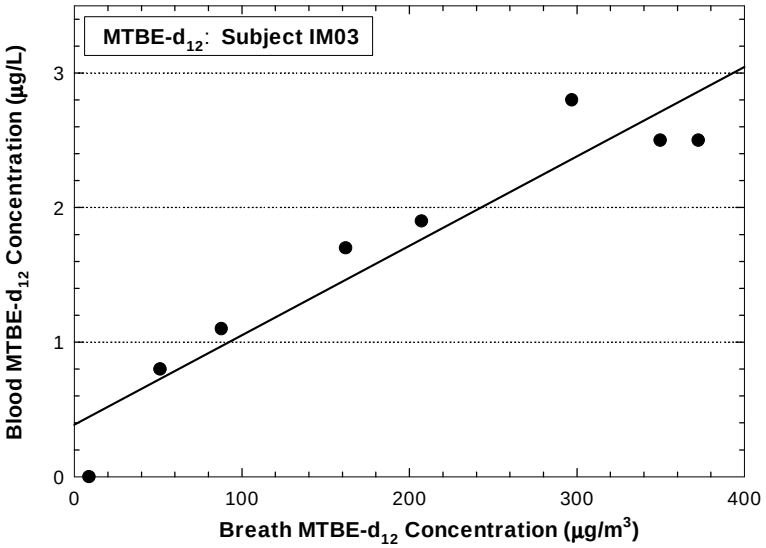

Figure 5-36. Measured breath MTBE-d_{12} concentrations vs. venous blood MTBE-d_{12} concentrations for male Subject IM03.

Table 5-5. Correlation between blood and breath concentrations and average blood:breath ratio for each participant.

Subject	Correlation Coefficient R^2	Average Blood:Breath Ratio
IF02	0.07	—
IM03	0.90	6.6
IM04	0.57	8.5
IM05	0.76	11.4
IM08	0.56	3.8
IF06	0.29	—
IM01	0.77	3.0
	Average:	6.7 ± 3.4

Quality Control Data

Exhaled Breath and Whole Air

The determination of the precision of a continuous real-time system, such as the breath inlet/glow discharge/ion trap combination, is not well defined but, using a reasonably constant source such as an environmental chamber, the variation in ion signal with time can be measured. By averaging the results over a suitable time period, values of the means and standard deviations for the target compounds can be found, to provide an overall measure of system stability and reproducibility. Figure 5-37 shows the time course of the average signal for the MS/MS fragment ion at m/z 55, obtained from a calibration standard of 2-butanone that was prepared in a 186-L glass chamber at a level of 866 μg/m^3 in zero-grade air. The ion current was sampled every 6 s and, at fixed intervals, the signal was averaged for 5 min. The ion intensity is almost constant over a 3½-h period, with a relative standard deviation of only 2.2%.

Quality control measures implemented in this study also included determining background levels and limits of detection for the compounds of interest. Background levels were estimated for the real-time breath measurements by passing humidified ultra-high purity air through the entire breath analyzer and measuring the signals at the masses used to monitor the target compounds. For the inhalation exposure study, the mean background levels for MTBE-d_{12} at m/z 82 and DBCM at m/z 129 were below the limits of detection, which were estimated by taking three times the standard deviation of the background (blank) mean concentration.[51] For

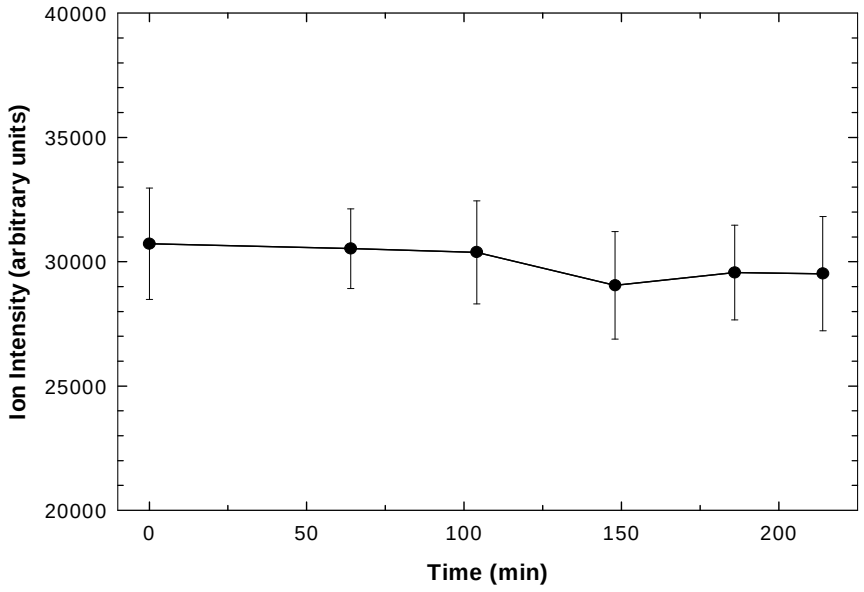

Figure 5-37. Plot of average ion signal (and standard deviation) at m/z 55 as a function of time, obtained from constant source of 2-butanone in glass chamber at a concentration of 866 μg/m^3 in zero-grade air.

Table 5-6. Limits of detection for MTBE-d_{12} and DBCM in exhaled breath, blood, and urine, and for TBA in blood and urine.

	Breath (µg/m^3)		Blood (µg/L)			Urine (µg/L)		
Subject	**MTBE-d_{12}**	**DBCM**	**MTBE-d_{12}**	**DBCM**	**TBA**	**MTBE-d_{12}**	**DBCM**	**TBA**
IF02	22.5	106.6						
IM03	14.5	44.8						
IM04	16.9	55.4						
IM05	17.4	47.8						
IM08	23.5	44.3						
IF06	18.0	41.3						
IM01	16.4	42.5						
Average	**18.5**	**46.0**[a]	**0.30**	**0.52**	**0.25**	**0.017**	**0.035**	**0.032**
SD (%RSD)	**3.3 (18)**	**5.1 (11)**[a]						

[a] Excludes value obtained for Subject IF02.

MTBE-d_{12}, the detection limits averaged 18.5 ± 3.3 (SD) µg/m^3; for DBCM, the average detection limit was 46.0 ± 5.1 µg/m^3. The detection limits for the real-time breath analyzer as well as for the blood and urine measurements are summarized in Table 5-6.

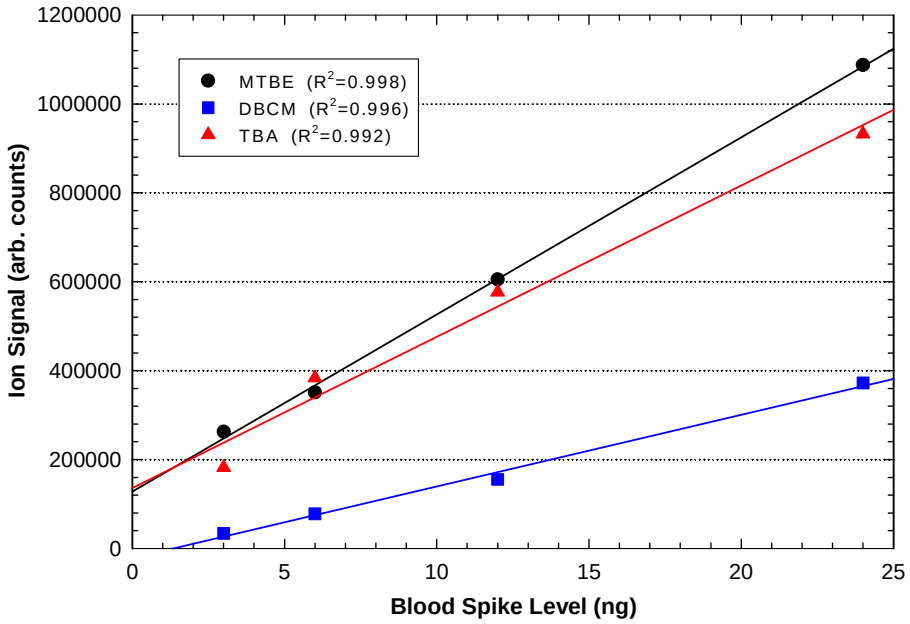

Figure 5-38. GC/MS ion signal response as a function of spike level of target compounds in blood.

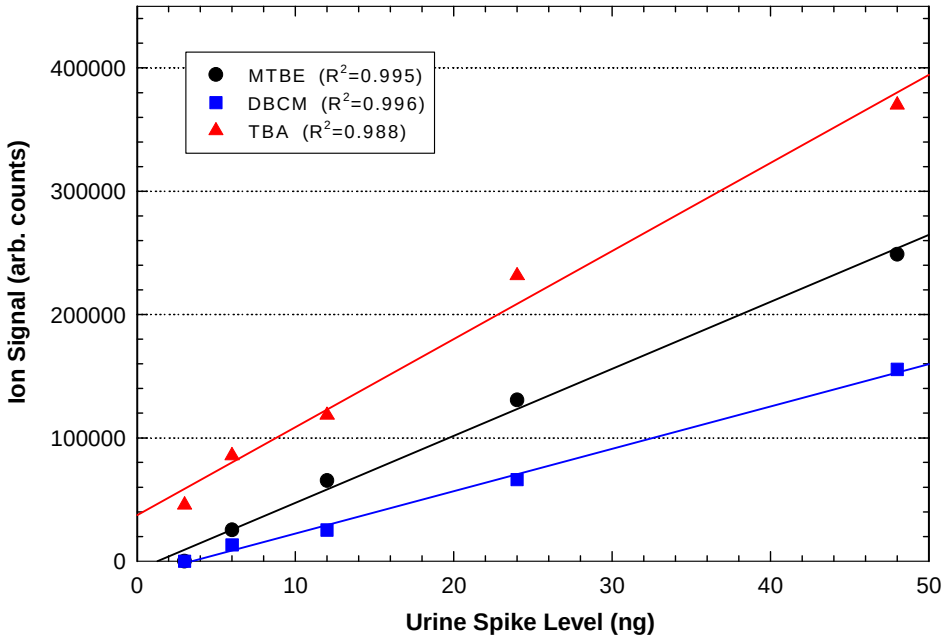

Figure 5-39. GC/MS ion signal response as a function of spike level of target compounds in urine.

Blood and Urine

Figures 5-38 and 5-39 show the calibration curves obtained for the target compounds in blood and urine, respectively. Blank blood samples were spiked at 4 levels, 3, 6, 12, and 24 ng; urine samples were spiked at five levels, namely, 3, 6, 12, 24, and 48 ng.

Chapter 6
Discussion

Breath and Blood Concentration/Time Profiles

For MTBE-d_{12}, the plots in Figures 5-15 to 5-21 show that exposure of the subjects to a constant level of 2,217 $\mu g/m^3$ (542 ppbv) for 30 minutes resulted in a rapid increase in the measured breath concentration from pre-exposure levels of 10 — 20 $\mu g/m^3$ (2 — 5 ppbv) to 200 — 450 $\mu g/m^3$ (50 — 110 ppbv). After exposure cessation, excretion resulted in a somewhat slower decrease in the breath levels, coming close to pre-exposure baseline levels after about 60 minutes. Background levels for MTBE-d_{12} in the exhaled breath were below the limit of detection, which was estimated by taking three times the standard deviation of the background concentration. For MTBE-d_{12}, the detection limits averaged 18.5 ±3.3 (SD) $\mu g/m^3$ (range 14.5 – 23.5 $\mu g/m^3$).

As explained earlier, uptake concentrations in the breath were determined by interrupting the exposure for brief periods and measuring the breath levels while the subjects breathed pure air. To minimize the deleterious effects of the signal noise, the breath data in the elimination phase were first smoothed using a 5-point (22.6-sec) moving average.

Subjects also were exposed to 728 $\mu g/m^3$ (85.6 ppbv) of DBCM for 30 minutes. Detection limits for DBCM were much higher than for MTBE-d_{12}, averaging 46.0 ±5.1 $\mu g/m^3$ (range 41.3 – 55.4 $\mu g/m^3$, excluding Subject IF02). By and large, background levels for DBCM in the exhaled breath were below the limit of detection, and the signal measured for this compound at m/z 129, the most abundant ion in the glow discharge mass spectrum, was exceptionally "noisy". The average signals during the uptake phase provided initial (pre-exposure) breath concentrations that ranged from 70 to 160 $\mu g/m^3$ and rose to between 130 and 250 $\mu g/m^3$ after 30 minutes. The high initial breath concentrations suggest that the measured signal at m/z 129 was probably elevated due to an unknown contaminant with fragment ions at the same mass. As noted earlier, we were unable to measure DBCM in the MS/MS mode because none of the precursor masses examined (m/z 127, 129, and 131) fragmented by collision-induced dissociation in the glow discharge/ion trap mass spectrometer.

Generally, the measured MTBE-d_{12} blood concentrations followed the same behavior as the measured breath profiles, but in a few cases, the correlation between blood and breath values was found to be quite poor, probably the result of significant and ongoing problems that were experienced at EOHSI with the analyses of the blood samples from this study.. As a result, we cannot place great store by the results obtained for the blood samples. Interestingly, the general shapes of the uptake portions of the blood and breath concentration/time plots for MTBE-d_{12} do

not suggest a slower response in the blood compared with the breath; however, the decay curves for the blood appear to return towards the baseline at a slower rate than do the breath curves.

The mean background level for MTBE-d_{12} in pre-exposure breath was 18.5 ± 3.3 (SD) μg/m^3 (range 14.5 – 23.5 μg/m^3), which was below the limit of detection of the real-time breath analyzer. This concentration is higher than that obtained in studies that made use of batch collection and analysis techniques to collect breath samples. In the single breath canister study conducted by Lindstrom and Pleil,[7] the pre-exposure breath levels ranged from 5.6 to 7.8 μg/m^3, whereas in the study conducted by Buckley et al.,[12] the background breath levels for one female and one male were 3.6 and 12.6 μg/m^3, respectively. In the more recent inhalation study reported by Lee et al.,[15,16] the mean pre-exposure breath level was 2.9 ± 4.3 μg/m^3. The mean pre-exposure blood concentrations of MTBE-d_{12} and TBA were 0.30 and 0.25 μg/L, respectively. Similar levels were measured by Moolenaar et al.[9] and Lee et al.[15,16]

Breath and Blood Residence Times

The models were fitted to the breath and blood uptake and decay data for MTBE-d_{12} for each subject. The results are shown in Figures 5-22 to 5-35, and values obtained for the calculated model parameters are summarized in Table 5-3.

The breath uptake data were consistent with a one-compartment model, the adjusted coefficients of determination (Adjusted R^2) associated with the model fits ranging from 0.85 to 0.97. The mean value for the one-compartment uptake residence times $\tau_{1uptake}$ was 5.7 ± 2.4 (SD) min (range 3.3 – 9.8 min). In contrast, the breath decay phase data gave satisfactory two-compartment fits; in this case, the adjusted R^2 values lay between 0.80 and 0.98. The mean value for the first compartment decay residence times τ_{1decay} was 3.8 ± 1.9 (SD) min (range 2.4 – 7.8 min); for the second compartment, the mean decay residence time τ_{2decay} was 61 ± 11 (SD) min (range 46 – 73 min). Table 6-1 shows that these breath decay values are generally consistent with values for the decay half-lives reported in previous studies. As noted earlier, it has been found that the residence times for many VOCs for the first two compartments are roughly similar, namely, 3 — 11 min for the first compartment, and 24 — 96 min for the second compartment. Our values fall within these ranges and are also in very good agreement with values reported in the literature (Table 6-1).

The blood uptake data were also consistent with a one-compartment model and were convergent in almost all cases. The associated adjusted R^2 ranged from 0.50 to 0.96 for the blood data, and the average blood residence time was essentially the same as that for the breath.

The quality of the blood decay data were such that we were only able to extract meaningful information from 2 or 3 data sets. Consequently, the results presented in Tables 5-3 and 6-1 must be treated with caution. Our values listed for the first and second blood residence times are similar to the values reported by Buckley et al.[12] The values reported by Lee et al.[15,16] for the first blood compartment and the second breath compartment prompted them to speculate that the second breath compartment rather than the first breath compartment is associated with a

Table 6-1. Summary of results obtained in current and previous MTBE exposure studies.

	Nihlén et al.[13,14]	Cain et al.[52]	Lindstrom and Pleil[7]	Buckley et al.[12]	Lee et al.[15,16]	This Study
Scenario	Chamber	Chamber	Gas station	Chamber	Chamber	Gas cylinder & face mask
Exposure	5 – 50 ppm, 2 h	1.7 ppm, 1 h	0.114 ppm, 2 min	1.39 ppm, 1 h	1.7 ppm, 15 min	0.542 ppm, 30 min
No. of Subjects & Gender	10 males	4	2	1 male, 1 female	3 males, 3 females	5 males, 2 females
Total Absorbed Dose (μg)					160 ± 50	148 ± 34
Fraction *f* Eliminated Unchanged			~0.7	0.60; 0.46	0.33 ± 0.06	0.29 ± 0.04
Fraction Absorbed	0.42 – 0.49				0.66 ± 0.06	0.73 ± 0.04
Fraction Exhaled Post-Exposure	0.32 – 0.47				0.40[a]	0.37 ± 0.06[b]
$\tau_{1uptake}$ (breath) (min)						5.7 ± 2.4
τ_{1decay} (breath) (min)			4.2	3.3; 1.7	3.0; 3.0	3.8 ± 1.9
τ_{2decay} (breath) (min)			49	53; 14	40; 35	61 ± 11
τ_{3decay} (breath) (min)				815; 190	421; 444	
$\tau_{1uptake}$ (blood) (min)						5.5 ± 3.9
τ_{1decay} (blood) (min)	1.2 – 1.6	58		5.2; 16	25; 54	5.5 – 19
τ_{2decay} (blood) (min)	12 – 17			61; —	82; 107	83; 114
τ_{3decay} (blood) (min)	121 – 139			1,904; 190	2,871; 1,284	
τ_{4decay} (blood) (min)	1,472 – 1,818					
Blood/Breath Ratio	17.7			18; 18	23.4; 23.6	6.7 ± 3.4

[a] Exhaled in 60 min after exposure.
[b] Exhaled in 90 min after exposure.

blood compartment. Neither our data nor that of Buckley et al.[12] provide support for this suggestion.

As noted earlier, the overall quality of the breath and blood uptake and decay data for DBCM adversely affected the results obtained for the residence times by applying the linear compartment model (cf. Table 5-4). In the case of the uptake residence time, τ_{uptake}, only one sample set (for Subject IF06) yielded a meaningful result, viz., $\tau_{uptake} = 2.2$ min. For the decay phase, meaningful results were obtained from three sample sets (for Subjects IM04, IM08, and IF06); the mean first-compartment residence time, τ_{1decay}, was 4.0 ± 2.4 min (all values highly significant). However, only one of the values obtained for the second compartment, τ_{2decay}, was significant, viz., 333 min (with $p < 0.05$ for Subject IM08).

Total Absorbed Dose and Fractional Uptake of MTBE

The total absorbed (internal) dose to a subject, defined as the amount of the chemical that passes through an absorption barrier or exchange boundary, is obtained from the difference between the total ("applied") dose and the "unmetabolized mass" (see Equation (4-10)). The mean MTBE-d_{12} total absorbed ("internal") dose was 149 ± 34 μg for the average 30-min exposure and a mean total ("applied") dose of 209 μg. The mean fraction of MTBE-d_{12} absorbed, or relative uptake, was 0.73 ± 0.04. For comparison, the 15-min exposure conducted by Lee et al.[15,16] resulted in a similar relative uptake of 0.66 ± 0.06.

Fraction *f* Exhaled at Equilibrium and Respiratory Fraction Eliminated Post-Exposure

The mean value for *f*, the fraction of the MTBE-d_{12} exposure concentration exhaled unchanged was 0.29 ± 0.04. This value is in good agreement with the value reported by Lee et al.,[15,16] and both are significantly lower than the values reported by Lindstrom and Pleil[7] and Buckley et al.[12]

The fraction of MTBE-d_{12} eliminated through expiration post-exposure was calculated from the ratio of the amount exhaled post-exposure (i.e., product of decay curve and alveolar ventilation rate) to the total absorbed dose. The mean fractional amount expired 90 minutes after exposure for all seven subjects was 0.37 ± 0.06. For comparison, Lee et al.[15,16] reported a mean value of 0.40 for 60 minutes after exposure.

Linear Compartment Coefficients

The coefficients of the exponential terms in Equation (4-15) determined from the MTBE-d_{12} breath decay data for the subjects are summarized in Table 5-3. For a constant exposure concentration, they provide a measure of the fraction of body burden in each compartment at equilibrium. This fractional contribution from each compartment depends upon the exposure period.[29,50] Thus, a relatively short exposure (e.g., 15 — 30 minutes) would not be expected to

result in significant transfer to the slower compartments, and most of the contribution would be from the first and, to a lesser extent, the second compartment. In this study, the exposure duration was 30 min, and post-exposure monitoring was limited to 60 — 90 min. Consequently, we assume that compartments 1 and 2 were fully equilibrated.

For the post-exposure period, the contribution of the i^{th} compartment is given by $a_i/\Sigma a_i$. The mean value for a_1 across the seven subjects was 0.57, and it was 0.43 for a_2, i.e., about 57% of the exhaled MTBE-d_{12} was associated with the blood compartment.

Blood/Breath Ratios

By and large, the measured blood MTBE-d_{12} concentrations correlated reasonably well with the breath concentrations, and the overall trends observed were generally consistent with previous studies.[12,15,16] However, peak levels, which ranged from about 0.9 — 2.5 µg/L, were significantly lower than those reported in other studies. Lee et al.[15,16] exposed 6 subjects to 1.7 ppm MTBE for 15 minutes and observed peak levels at the end of exposure which ranged from 4 to 10 µg/L. For two subjects exposed to 1.39 ppm for 1 h, Buckley et al.[12] observed peak blood levels of 8.7 and 15 µg/L. Much higher peak MTBE levels were reported by Nihlén et al.,[13,14] who exposed 10 subjects to 5 — 50 ppm for 2 h and obtained levels between 123 and 1,144 µg/L. Despite the low applied dose used in this study, the plot in Figure 6-1 shows a strong

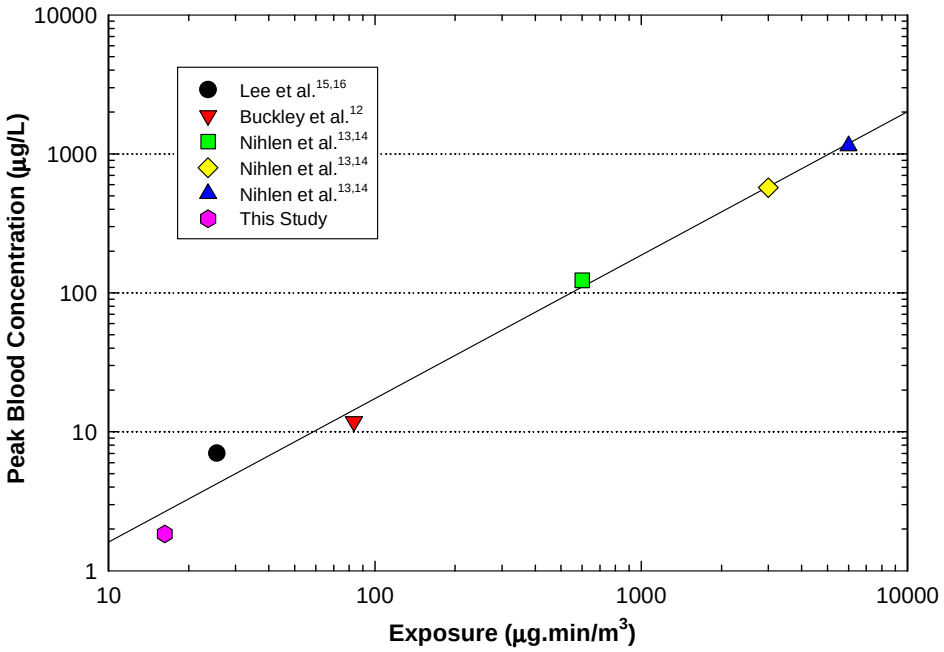

Figure 6-1. Dependence of mean peak blood concentration for MTBE-d_{12} on total ("applied") dose from this study compared to literature values.

linear relationship (R^2 = 0.987) between mean peak blood concentration and total ("applied") dose (exposure) from the different studies, including this one, over almost three orders of magnitude.

An example of the relation of blood to exhaled breath measurements of MTBE-d_{12} is shown in Figure 6-1 for Subject IM03, and summary statistics for all seven subjects are presented in Table 5-5. Using linear regression analysis, the mean blood/breath ratio was found to be 6.7 ± 3.4. Nihlén et al.[13,14] obtained a blood/breath partition coefficient of 17.7, Buckley et al.[12] reported a ratio of 18, and Lee et al.[15,16] found a ratio of 23.5. The reason for the significantly lower value calculated in the present study is not clear. We speculate that it may have been due to a number of problems that were experienced in the course of the analysis of the blood samples in the laboratory. Because of these problems, the analyses took more than 6 months to complete, a period that was far in excess of the normally accepted storage period for a VOC such as MTBE in blood samples. The low measured blood concentrations may well have been due to losses of MTBE that occurred during this period.

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